Encyclopedia of Pathology
Series Editor: J.H.J.M. van Krieken

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Fernando Schmitt *Editor*

Cytopathology

Springer

Encyclopedia of Pathology

Series Editor
J. H. J. M. van Krieken

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Fernando Schmitt
Editor

Cytopathology

With 649 Figures and 15 Tables

Springer

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Dedicated to Els, Lotte, Bas and Wouter who are my inspiration

J.H.J.M. van Krieken

Series Preface

When Denis Diderot started the first encyclopedia in the eighteenth century, it was a groundbreaking and timely event. It was the time of the Enlightenment, and knowledge was seen as something which was to be spread to many and to build upon by creating new knowledge. His ambition was to bring all available knowledge together in one series of books so that every person who could read has access to all there is to know. Nowadays, in a time of easily accessible knowledge, the question is whether there is still need of an encyclopedia. It is obvious that the amount of knowledge is such that it is not possible to bring it all together in one encyclopedia. One may argue that the Internet is the encyclopedia of today, but that misses an important point of Diderot, a point that is probably even more valid today. He created a team that valued information and selected what was worth to be presented in the encyclopedia. He recognized that science is not a democratic process where the majority decides what is true and valuable, but rather a growing body of knowledge in which radical ideas from individuals may bring about huge changes, even though most would reject these new ideas in the beginning. Indeed, the Internet lacks such authority and it is not easy to select valuable information from nonsense, especially when one is not an expert in a certain field.

It is therefore that an encyclopedia is only as good as the team that creates it. It goes without saying the team that is responsible for the *Encyclopedia of Pathology* consists of recognized experts in the field. Pathology is a growing medical discipline in which the amount of information is probably already more than that the whole encyclopedia of Diderot contained. For experts in subspecialties within pathology, it is already almost impossible to keep an overview on new developments and to select relevant from less relevant new information. There are plenty of textbooks for every disease group, and scientific literature is available for most pathologists through PubMed or Google Scholar. What is lacking is a systematic overview of what we know in an alphabetical order, easily accessible to all. The encyclopedia of pathology fills that gap. It is written by experts with the general pathologist in mind and also specialist from other disciplines. It will consist of a series of volumes on subspecialties, and when it is completed there will be an online version combining these. Yearly updates from the online version is foreseen and readers are welcome to provide suggestions for improvement. These will be judged by the editorial team in order to keep the encyclopedia authoritative yet using the expertise of many.

Finally, it is my hope that the encyclopedia will grow into a reliable body of knowledge in pathology, enabling communication through a common language, and that it will grow and adapt to new developments.

Nijmegen, The Netherlands J.H.J.M. van Krieken
March 2017

Volume Preface

The practice of cytopathology has undergone significant evolution in the last 50 years. Cytopathology is actually considered a diagnostic method more than a screening method in most of the areas that it is applied and is an integral part of pathology. Furthermore, in the last years the incorporation of new technologies on cytology material, as molecular techniques, has been essential in driving classification of tumors and providing prognostic and predictive information. However, when more we advance for the molecular diagnostic more we observe the value of a good and precise morphological evaluation of the specimens. In this encyclopedia, we cover all of main areas where a cytological interpretation/diagnosis is possible. There are a total of 210 chapters, covering different pathological entities, revising clinical and cytomorphological aspects, as well as with comments about ancillary techniques, including immunocytochemistry and molecular findings. The chapters are well illustrated with representative pictures that will help the reader that uses the encyclopedia. Most of the more experienced cytopathologists around the world contributed for this book bringing their personal professional and teaching experience. This will be a great value for the general, specialist, and for the trainee pathologists in their daily practice. I would like to thank personally all the contributors of this encyclopedia for their time and availability in sharing their knowledge with our readers. Knowledge in medicine is advancing very fast in different scientific domains. Image guidance, less invasive procedures, and availability of cells or tissues from patients are crucial for disease diagnosis, identification of molecular targets, and study of prognostic factors. This makes cytology to have a central role in modern medicine. The dynamic aspect of the encyclopedia, as a living platform that can incorporate updates and incorporate new entries in an ongoing basis, fits perfectly with the current speed of creation of new knowledge in medicine, pathology, and cytopathology. I hope that this encyclopedia can serve our readers to obtain helpful information for better diagnosis and management of the patients. Now it is time to enjoy the text and the illustrations of this book that presents the up-to-date knowledge in cytopathology.

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Acknowledgments

Pathologists serve everyday new patients based on the knowledge collected in this encyclopedia gained over more than a century from other patients and pathologists. I am therefore feeling a deep gratitude to all of them. I also like to thank all who have contributed to the large amount of items but especially to the editors who had the difficult task to select and collect, evaluate and approve.

J.H.J.M. van Krieken

Editor Biography

Fernando Schmitt is professor of pathology at the University of Porto, senior researcher at IPATIMUP, and director of the Department of Medicine and Pathology at Laboratoire National de Santé in Luxembourg.

Professor Schmitt has authored more than 436 papers in peer-reviewed journals, 23 book chapters, and two books; he is widely considered a world-leading expert in cytopathology and breast cancer. Professor Schmitt was a Fellow at the Karolinska Medical Hospital, Stockholm, and was professor of pathology at the University of São Paulo, Brazil, and University of Toronto, Canada. His main research work is on breast cancer, with emphasis on molecular markers, cell adhesion and invasion, as well as on therapeutic targets and mechanisms of resistance. Professor Schmitt is also dedicated to the research and use of molecular techniques on cytological material. Professor Schmitt serves as associate editor of six major scientific journals and sits on the editorial board of many others. He has also been chairman, president, and scientific director of several major European, Portuguese, and Brazilian scientific societies. Currently, he is the secretary-general of the International Academy of Cytology (IAC). Professor Schmitt received the prize of Educator of the Year 2011 by the Papanicolaou Society of Cytology (PSC) and also received the prize GOLDBLATT AWARD 2013 from the International Academy of Cytology.

Series Editor Biography

J.H.J.M. van Krieken is a pathologist with special expertise in the fields of hematopathology and the pathology of the gastrointestinal tract. He was professor for tumor pathology since 1999 and kept from 2005 to 2015 the chair of pathology at the Radboud University Nijmegen Medical Centre in Nijmegen. He furthermore served as chairman of the Board of the Oncology Institute of the Radboud University Nijmegen from 2008 to 2016. Since 2016, he is the rector magnificus (vice chancellor) of the Radboud University.

He was the treasurer/secretary of the European Association for Hematopathology from 2000 to 2008, from 2003 to 2011 the treasurer, from 2013 to 2015 the president of the European Society for Pathology (ESP), and from 2015 to 2017 the past-president of the ESP. Furthermore, he coordinates the ESP quality assessment program and is the chair of IQNpath. He is (co) author of more than 500 papers in peer-reviewed journals (H-index 79), has written chapters in books on pathology and oncology, is editor of a Dutch textbook on oncology, and serves on the editorial board of the *American Journal of Surgical Pathology*, is managing editor of *Virchows Archiv*, and is the chief editor of the *Journal of Hematopathology*. Since 2011, he is member of the German Academy of Sciences Leopoldina, and since 2014 of Academia Europea and Honorary Fellow of the Royal Society of Pathology of Great Britain and Ireland.

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A

Acinic Cell Carcinoma, Cytological Findings

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Synonyms

Acinic cell adenocarcinoma. The term acinic cell tumor is no longer appropriate.

Definition

Acinic cell carcinoma (AcCC) is one of the three most common primary salivary malignancies with adenoid cystic carcinoma and mucoepidermoid carcinoma (Barnes et al. 2005; Ellis 2008). AcCC is an adenocarcinoma showing acinic cell differentiation with zymogen-type secretory granules. The new entity called mammary analogue of secretory carcinoma (MASC) was described in 2010 (Skálová et al. 2010).

Clinical Features

- **Incidence**
  AcCC represents approximately 3% of salivary gland tumors and 8% of malignant major salivary gland tumors.
- **Age**
  AcCC may occur at any age (including pediatric age), and the peak of incidence is localized between 20 and 70 years.
- **Sex**
  A slight female incidence is observed.
- **Site**
  The parotid gland is the predominant (80%) site and around 10% arise in the minor salivary glands.
- **Treatment**
  Surgery is usually an initial treatment of AcCC.
- **Outcome**
  Recurrences are possible. Metastatic evolution is rare.

Macroscopy

AcCC consists in a well-circumscribed, solitary nodule with irregular borders with solid and cystic areas. AcCC may be well, moderately or poorly differentiated. The morphologic growth patterns are solid, microcystic, papillary cystic, and follicular-like (Fig. 1).

Microscopy

Smears in AcCC are usually hypercellular and cell-rich and stroma-poor (Klijanienko and Vielh 1997a). AcCC belongs to the group of tumors exhibiting predominant epithelial cell morphology.

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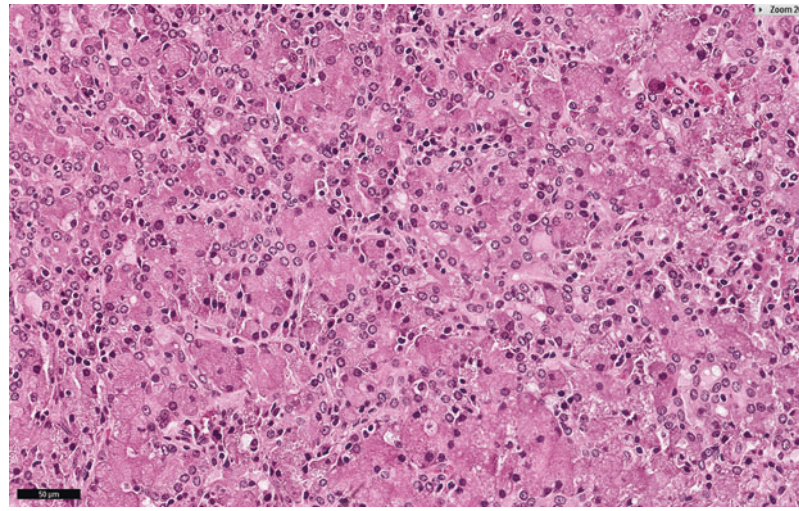
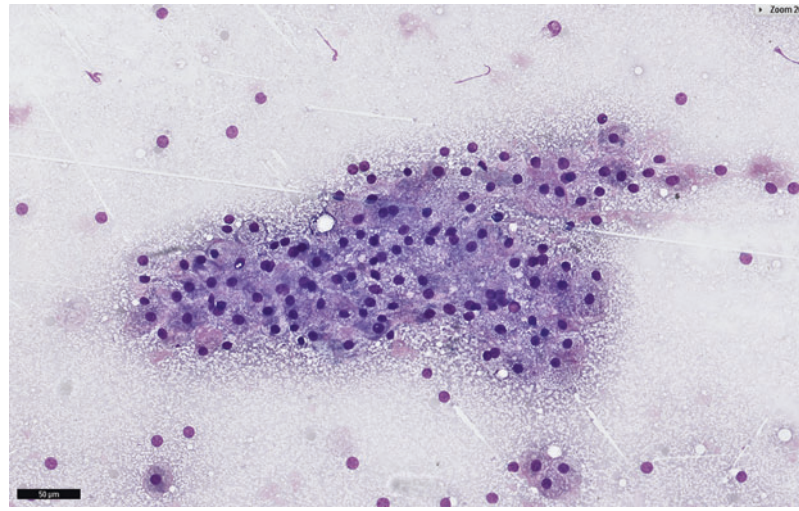
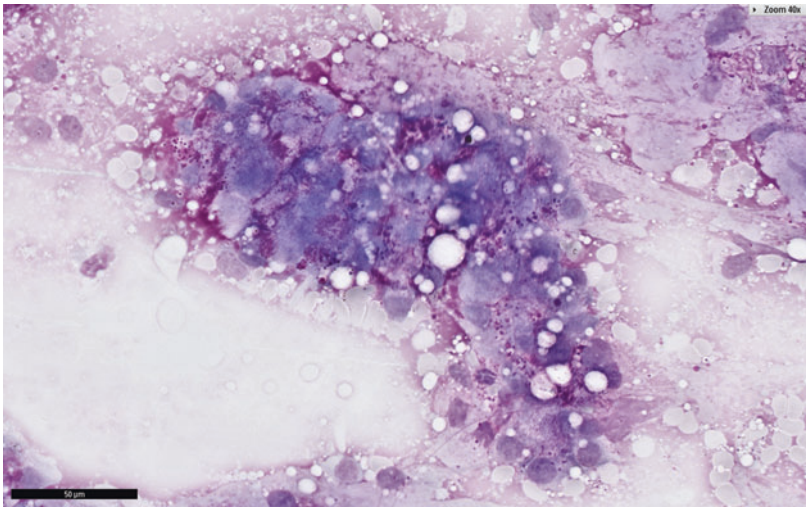


Acinic Cell Carcinoma, Cytological Findings, Fig. 1 Acinic cell carcinoma. Solid proliferation of acinic cells. Some lymphocytes are also visible (H&E stain)



Acinic Cell Carcinoma, Cytological Findings, Fig. 2 Acinic cell carcinoma, well differentiated. Tumor cells are similar to normal acinic cells from the salivary glands. Note the presence of naked nuclei (MGG stain)

Cytological diagnosis of AcCC depends on cellular differentiation, i.e., well, moderately, and poorly differentiated. Accurate diagnosis is based on the recognition of clustered (sometimes in pseudopapillary structures) malignant acinic cells. When the tumor is well differentiated, cells are very similar to normal acinic cells from the salivary glands. Cells are large and micro-vacuolated and show small, round, and central nuclei (Fig. 2). When the tumor is moderately differentiated, cells are polyhedral with eccentric small nuclei and abundant blue-gray micro-vacuolated (clarified) cytoplasm. Nuclei are larger. When the tumor is poorly differentiated, cells exhibit nonspecific adenocarcinomatous pattern with large, atypical nuclei. Moreover, whatever the differentiation, numerous naked nuclei are usually present in the background. Some tumors may show lymphocytes. Necrosis and mitotic figures are absent. Oncocytic-like cells, dark cuboid cells, round cells, and cohesive cells with azurophilic granules (MGG) may be seen and are



Acinic Cell Carcinoma, Cytological Findings, Fig. 3 Acinic cell carcinoma. Zymogen-type secretory granules appearing *magenta-violet* using MGG stain (MGG stain)

pathognomonic for AcCC (zymogen-type secretory granules) (Fig. 3). Some cases may contain intracytoplasmic crystalloids or psammoma bodies.

Special attention should be done in cases without visible secretory zymogen granules and/or cases of "papillary cystic" or "follicular-like" variants of AcCC which may correspond to MASC (Skálová et al. 2010; Pisharodi 2013; Bishop et al. 2013).

Immunophenotype

Immunohistochemistry is not specific, and immunoreactivity with cytokeratin, lactoferrin, α1-chymotryspin, and amylase is known for AcCC. Conversely, the immunoprofile of MASC with frequent expression of S-100 protein, mammaglobin, and vimentin is very rare in AcCC.

Molecular and Cytogenetic Features

Cytogenetic alterations in AcCC involving chromosomes 4p, 5q, 6p, and 17p have been described. MASC harbors a t(12;15)(p13;q25) translocation fusing the ETV6 transcriptional regulator to the NTRK3 membrane receptor kinase. This fusion is identical to that found in human secretory breast carcinoma as well as in congenital fibrosarcoma and cellular mesoblastic nephroma and is detectable by FISH (Weinreb 2013).

Differential Diagnosis

AcCC should be differentiated from sialadenosis but also from epi-myoepithelial carcinoma (EMEC) and oncocytoma/Warthin's tumor (WT). Well-differentiated AcCC may be extremely difficult to differentiate from sialadenosis, by the abundance of acinic cells. Bilateral lesion, association with heavy pathology (diabetes, cancer), lack of naked nuclei, and irregular aspect of clusters help in the differential diagnosis (Klijanienko and Vielh 1997a; Gupta and Sodhani 1998). EMEC may contain some clear cells and the differential diagnosis is relatively easy. Cytomorphological features of EMEC include three-dimensional cellular aggregates with clear cytoplasm at the periphery and presence of stromal connective components like tubular structures and hyaline globules which are absent in AcCC (Klijanienko 1997; Klijanienko and Vielh 1998). Some WT and oncocytomas may be composed of oncocytic cells with microvacuolated cytoplasm. Both entities may be excluded by the presence of clarified cells arranged in acini and numerous naked nuclei, usually characteristic for AcCC (Klijanienko and Vielh 1997b).

Finally, AcCC should be differentiated from a metastasis from renal cell carcinoma (Mrena et al. 2008).

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Acute Thyroiditis, Cytological Findings

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Synonyms

Thyroid infectious disease; Viral thyroiditis

Definition

Acute inflammation of the thyroid gland usually due to microorganisms.

Clinical Features

- **Incidence**
  It is currently an uncommon and rare process.
- **Age**
  Observed either by teenagers or immunocompromised adult people.
- **Sex**
  May occur whatever the gender.
- **Site**
  The lesion may be found anywhere in the thyroid gland.
- **Treatment**
  Treatment is based on a targeted antibiotic therapy and in some cases on abscess drainage.
- **Outcome**
  Usually there is no adverse outcome, except in severe immunocompromised hosts.

Macroscopy

These lesions should not be treated by surgery and less invasive treatment would be more relevant. Nevertheless, in case of surgical management, a wide thyroid increase could be observed with edema and eventually abscess and cavitation due to abscess partial or total drainage.

Microscopy

Fine-needle aspirations (FNA) are rarely recommended in acute thyroiditis since the diagnosis is usually based on clinical symptoms alone, which are pain and swelling of the gland with more or less fewer and inflammatory signs. Nevertheless, an FNA can be discussed in order to eliminate a diagnosis of malignant involvement. It is then possible to observe the following on the slides: (1) scant colloid or no colloid; (2) numerous granulocytes, sometimes well preserved, in other cases as pyocytes; (3) necrosis and proteinaceous material in the background; (4) more rarely microorganisms, mostly Gram-positive bacteria, and sometimes microorganisms like aspergillums, Cryptococcus, *Pneumocystis carinii*, *Candida albicans*, or virus inclusions, especially in immunocompromised hosts (in these patients, culture, Gram, and specific stainings are helpful); and (5) follicular cells may be observed, usually sparse and quite atypical due to some reactive changes.

Cytological criteria are the same in direct smears and in liquid-based cytology.

Immunophenotype

Immunophenotyping is not necessary except for an eventual virus typing.

Differential Diagnosis

Differential diagnosis essentially includes anaplastic carcinoma since both, anaplastic carcinoma and acute thyroiditis, share some similar symptoms clinically and very atypical cells on FNA; usually the cells are more atypical in anaplastic carcinoma than in acute thyroiditis, but there is a risk of a false positive in cases of low cellularity.

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Adenoid Cystic Carcinoma, Cytological Findings

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Synonyms

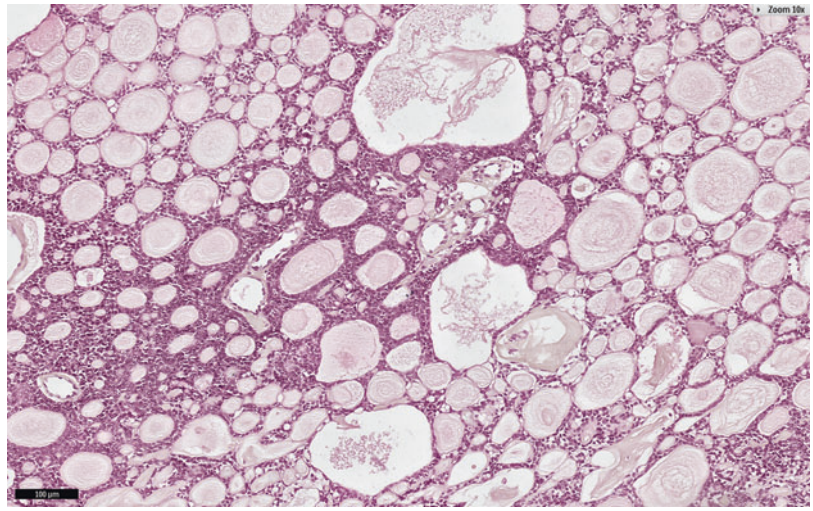
Cylindroma

Definition

Adenoid cystic carcinoma (ACC) (Barnes et al. 2005; Ellis and Auclair 2008) is a salivary adenocarcinoma of epithelial and myoepithelial differentiation: its characteristic cribriform architecture is the most frequent pattern (Fig. 1). Together with acinic cell carcinoma and mucoepidermoid carcinoma, ACC belongs to the most common primary salivary gland malignancies.

Clinical Features

- **Incidence**
  ACC represents approximately 10% of all salivary gland carcinomas and 2% of all salivary gland lesions.
- **Age**
  ACC arises in all ages, including pediatric age. Its peak of incidence is in the sixth decade of life.
- **Sex**
  There is an equal sex distribution.
- **Site**
  Major and minor salivary glands are affected in equal percentage of cases.



Adenoid Cystic Carcinoma, Cytological Findings, Fig. 1 Adenoid cystic carcinoma. Typical cribriform architecture (H&E stain)

- **Treatment**
  Surgical treatment with complete excision is usually an initial treatment.
- **Outcome**
  ACC may have recurrent and metastatic evolution.

Macroscopy

ACC presents as a firm gray to white poorly circumscribed mass. ACCs are categorized as cribriform, tubular, or solid, but usually they are mixed. Solid type with central necrosis is a high-grade malignancy.

Microscopy

Smears in ACC are usually hypercellular and cell-rich and stroma-rich. ACC belongs to the group of tumors exhibiting predominant myoepithelial cell morphology (Klijanienko and Vielh 1997).

Cells are basaloid, small, dark, isolated, or clustered. Usually a scant rim of cytoplasm is well seen. Chromatin is coarse with small nucleoli. Mild cytoatypia/nuclear atypia is common. Necrosis and mitotic figures may be seen in poorly differentiated (solid) ACCs (Klijanienko and Vielh 1997).

Stroma is characteristic and allows a proper cytologic diagnosis. Smears contain four characteristic stromal components: hyaline globules, tubular/cylindrical structures, fingerlike bowl-shaped structures, and acellular connective debris (Figs. 2 and 3) (Barnes et al. 2005; Ellis and Auclair 2008; Klijanienko and Vielh 1997).

Immunophenotype

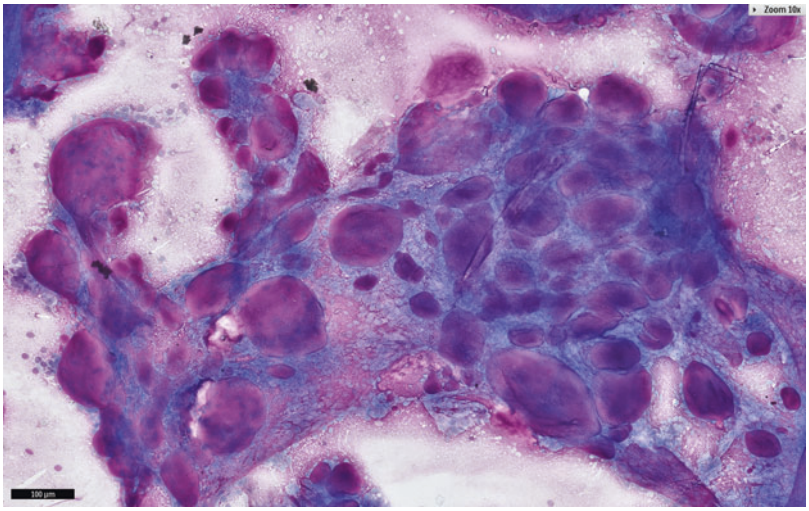
Epithelial cells are immunoreactive for cytokeratins 7, 14, 17, and 19, EMA, CEA, S-100, and CD-117 and myoepithelial cells for muscle-specific actin, p63, and calponin.

Molecular and Cytogenetic Features

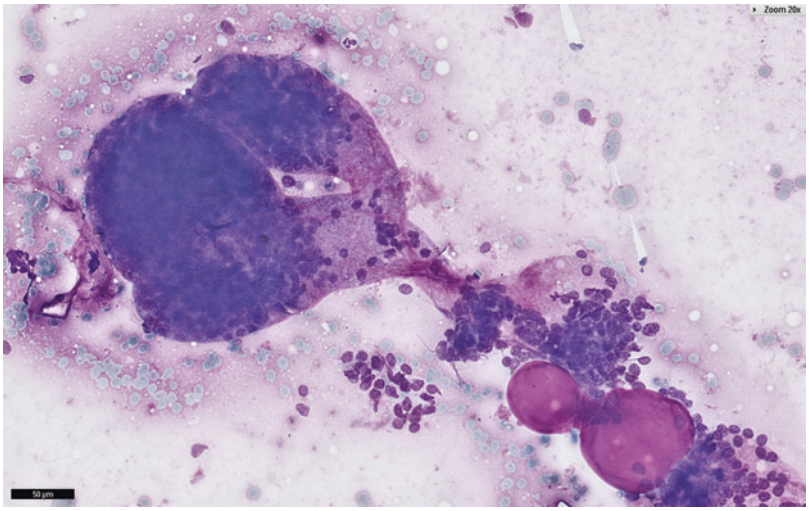
One third of tumors show deletion of 1p32–p36, and recurrent fusion between the MYB oncogene and NFIB transcription factor is frequent (Weinreb 2013).

Differential Diagnosis

The presence of round hyaline globules should not automatically lead to a diagnosis of ACC as these elements may be only occasionally seen in



Adenoid Cystic Carcinoma, Cytological Findings, Fig. 2 Adenoid cystic carcinoma. Numerous hyaline globules (MGG stain)



Adenoid Cystic Carcinoma, Cytological Findings, Fig. 3 Adenoid cystic carcinoma. Hyaline globules, tubular structures, and roundish fingerlike structures. Note the presence of basal, dark cells and dispersed naked nuclei (MGG stain)

these tumors. For these reasons, ACC should be differentiated from other salivary tumors showing predominant myoepithelial features: cellular pleomorphic adenoma (CPA), polymorphous low-grade adenocarcinoma (PLGA), and epi-myoepithelial carcinoma (EMEC), as well as basal cell tumors (adenoma/adenocarcinoma (BCA)). Correct recognition of plasmacytoid myoepithelial cells with distinctive cell borders in CPA is crucial in the differential diagnosis (Klijanienko and Vielh 1996). ACC lacks dispersed myoepithelial cells, while CPA usually lack naked nuclei, which are frequently seen in ACC. Moreover, ACC lacks chondromyxoid stroma which is constantly present in CPA.

PLGA is also similar to ACC and consists of various patterns ranging from solid to tubular, papillary, cribriform, or fascicular. The presence of clarified nuclei and specific palate localization is a precious help in the differentiating these two entities (Klijanienko and Vielh 1996).

EMEC may closely resemble ACC. It includes three-dimensional cellular aggregates of basaloid cells, with clear cells at the periphery. The presence

of basaloid cells and amorphous stromal substance may be reminiscent of ACC, but the presence of clear cells is distinctive (Klijanienko and Vielh 1998b).

The differentiation between BCA and ACC may also be difficult. Unlike ACC, BCA are composed of small, regular, and clarified epithelial basaloid cells with scanty cytoplasm and a bland nuclear chromatin. Clusters are three dimensional with peripheral palisading. Naked nuclei and nonspecific connective fragments are frequent. All these patterns are absent in ACC. Moreover, hyaline globules may be present in BCA and are usually seen in basal cell adenocarcinoma. Basal cell adenocarcinoma may be undistinguishable from poorly differentiated ACC, when fingerlike and tubular structures are absent on the smears (Klijanienko et al. 1999).

Finally, the differential diagnosis includes also non-salivary tumors at parotid localization such as dermal eccrine spiradenoma, cutaneous basal cell carcinoma, and basaloid squamous cell carcinoma from ENT area.

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Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

Adnexal tumors arise from skin adnexa, Meibomian and Zeis glands. Much of these tumors differentiate along one adnexal line, forming distinct morphological and phenotypical types. Differentiation along two lines in the same tumor, either benign or malignant, may occur. Most of the tumors are benign, but apocrine, mucinous, and signet adenocarcinoma and Meibomian or Zeis carcinoma may occur.

Clinical Features

- **Incidence**
  Infrequent, Meibomian and Zeis gland tumors more frequent in Asian countries.
- **Age**
  Middle-old age.
- **Sex**
  No gender predilection.
- **Site**
  Adnexal tumors – eyelids skin, Meibomian: the eyelids rim inside the tarsal plate. Zeis: the margin of the eyelids.
- **Treatment**
  Surgery, chemotherapy.

- **Outcome**
  Favorable for benign tumors. Variable for malignant adnexal tumors. Unfavorable in more than 25% of Meibomian carcinoma.

Description

Eyelid hidradenoma and eccrine poroma diagnosed by FNA have been reported. Hidradenoma has been described as a cystic tumor showing an amorphous background material with or without foam cells. The epithelial component is represented by duct-like cells and tubular structures. A biphasic cell population with both eosinophilic and clear to basophilic cells may be seen with the Papanicolaou stain. Eccrine poroma shows small sheets of cuboidal cells in a proteinaceous background. The cells were uniformly sized having round-to-oval nuclei with inconspicuous nucleoli and scant-to-moderate pink granular cytoplasm. The cytological features of adnexal, Meibomian, and Zeis gland tumors features are similar to those described in the skin.

Cross-References

- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Adrenal Myelolipoma, Cytological Findings

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Definition

Myelolipoma is an uncommon, benign, endocrinologically inactive tumor of unknown pathogenesis composed of adipose and myeloid tissue in varying proportions.

Clinical Features

- **Incidence**
  Most myelolipomas are small and asymptomatic and are found incidentally in a CT-Scan or at post-mortem examination. However a small percentage of the patients can have flank pain, hematuria, palpable mass or hypertention. Incidence is difficult to estimate. Bilateral tumors occur in about 10% of cases (Bennett et al. 1980). Adrenal myelolipoma occurs in 70% of patients as a de novo lesion (Kenney et al. 1998). However, it may be associated with endocrine disorders, such as Cushing's syndrome and, Conn's syndrome beyond others or in association with other adrenal tumors including cortical adenoma, carcinoma and pheochromocytoma (Bennett et al. 1980; Escuin et al. 1985; Whaley et al. 1985; Vyberg, M., & Sestof, L 1986; Goetz et al. 1994).
- **Age**
  Usually found in middle-aged patients ranging between 41 and 81 years (mean 61 years old).
- **Sex**
  Female/male ratio is of 2:1.
- **Site**
  Most tumors occur in the adrenal gland but extra adrenal sites have been referred. The most common extra-adrenal site is in the presacral region but other sites like mediastinum, liver, stomach, lungs, spleen, retroperitoneum, and mesentery are reported.
- **Treatment**
  Surgical excision is proposed for large and symptomatic lesions. If asymptomatic, CT scan follow-up is advised.
- **Outcome**
  This is a benign lesion with no metastatic potential.

Macroscopy

Most lesions are solitary and can vary in size, ranging from just few millimeters to as large as 34 cm. The tumor is well circumscribed, although not capsulated. The cut surface appearance varies according to the most predominant component, fat or myeloid elements appear with a tone which ranges from yellow to red or mahogany. Hemorrhage or necroses are common features.

Microscopy

In histology, islands of hematopoietic cells (myelocytes, erythrocytes, and megakaryocytes) and mature adipocytes are seen. In larger tumors, hemorrhage, necrosis, fibromyxoid degeneration, calcification, and cyst formation can also be seen. In adrenal lesions, there is usually compressed cortical tissue surrounding the tumor.

There are few cytological reports of this entity as most tumors are diagnosed by radiology. Cytologic smears are characterized by the presence of mature and immature hematopoietic elements, myeloid and erythroid cells mixed with mature adipose tissue fragments. The bone marrow elements observed are normal and in various stages of maturation reproducing the normal cellular maturation of bone marrow (Destouni et al. 2001).

Immunophenotype

Does not bring any contribution to diagnosis.

Molecular Features

There are no characteristic molecular changes in myelolipoma. However there are some reports of sporadic and inconstant molecular changes like t(3;12)(q27;q13), t(3;12) (q28;q14), and t(X;12) (q27;q14).

Differential Diagnosis

Differential diagnosis should be raised with angiomyolipoma, liposarcoma, and extramedullary hematopoiesis.

Fine needle aspiration of an angiomyolipoma can be selective showing only adipose tissue. Typical thick-walled vessels identified in histology of angiomyolipomas are hardly seen in the smears; no hematopoietic cellular elements are present and unlike myelolipomas, these lesions are HMB45 positive.

Lipoblasts and atypia that characterize liposarcoma are not present in myelolipomas that only show mature adipose cells.

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Adrenocortical Tumors, Cytological Findings

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Definition

Adrenal cortical tumors include adenoma and carcinoma. The adrenal cortical cell is a part of endocrine epithelia and has the capacity of hormone production: steroids, glucocorticoids, and mineralocorticoids. Both adenomas and carcinomas from the adrenal cortex can be hormone secreting. Adenomas are usually detected incidentally during a computerized axial tomography (CT scan) or any other image technique performed for other reason. Adenomas are rarely functional and cause endocrine syndromes in less than 15% of the patients. About 50% of carcinomas are hormonally functional being mostly associated whether with Cushing syndrome (glucocorticoid secretion) or virilization of women patients (androgen secretion).

Clinical Features

- **Incidence**
  Adenomas are rare and, in most instances, accidentally found. They are present approximately in 1–10% of persons at autopsy. Carcinomas are also rare accounting for 0.02% of all malignant neoplasms in humans.

- **Age**
  Adenomas are benign tumors and affect patients mainly after 30 years old. Adrenocortical carcinoma has a bimodal distribution by age, with cases clustering in children under 6 and in adults over 30 years old.
- **Sex**
  No sex predilection is found in adenomas or carcinomas.
- **Site**
  Adrenal cortex
- **Treatment**
  Adrenal cortical adenomas and carcinomas should be excised. Radio-frequency ablation may be used for palliation in patients who are no longer surgical candidates.
- **Outcome**
  Adenomas are benign tumors and can be cured after excision or can be monitored if incidental and very small. Carcinomas are aggressive tumors usually diagnosed in advanced stages. Tumor stage, **T**umor **M**etastases **N**odules (TMN), is the most important factor determining prognosis. 5-year survival rates for patients with stages I, II, III, and IV are of 66%, 58%, 24%, and 0%, respectively.

Macroscopy

Adenomas are small lesions measuring less than 5 cm large, well circumscribed, and solitary. The cut surface is solid, homogenous, and yellow. No hemorrhage or necrosis is present.

Carcinomas are large lesions weighing more than 100 g and have extensive necrotic and hemorrhagic areas. Not infrequently tumors extend beyond the capsule invading local structures, liver, or into the adrenal vein.

Microscopy

Histologically adrenal cortical tumors are classified as adenomas or carcinomas whenever they fill the Weiss criteria:

Original Weiss criteria for malignancy (Aubert et al. 2005)

1. Nuclear grade III or IV based on Fuhrman criteria
2. More than 6 mitotic figures/50 high-power field (40x objective), counting 10 random fields in an area of greatest number of mitotic figures on 5 slides with the greatest number of mitoses
3. Presence of atypical mitotic figures
4. Clear or vacuolated cells comprising 25% or less of tumor
5. Diffuse architecture in more than 1/3 of the tumor
6. Confluent necrosis
7. Venous invasion
8. Sinusoidal invasion
9. Capsular invasion (either incomplete or complete)

Note 1: The above criteria should not be applied to childhood tumors or to oncocytic tumors.

Note 2: More than three criteria indicate malignant potential.

Weiss criteria were recently modified, excluding all possible subjective criteria such as architecture, pleomorphism, and sinusoidal or venous invasion (Lau and Weiss 2009).

Difficulties distinguishing benign from malignant tumors persist, particularly in large tumors without invasive features and cellular atypia or in those who fall in the Weiss "grey zone" (Tissier 2010). In problematic cases, the term adrenal cortical tumor of uncertain malignant potential can be a solution.

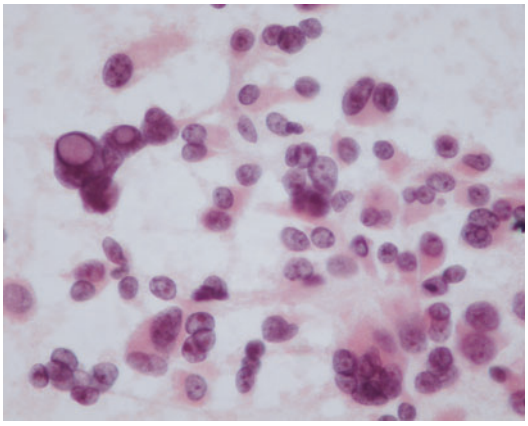
Adrenal cortical tumors reproduce, if well differentiated, the morphologic pattern of the normal gland with alveolar architecture, cords, or nests of cells separated by fine sinusoids. Neoplastic cells are polygonal with abundant bubbly or eosinophilic cytoplasm. Pleomorphism can be quite variable being more evident in carcinomas, but is not a criteria for malignancy. Similarly, mitotic count varies. Higher rates occur in carcinomas, and it is not infrequent to find atypical mitotic figures in these tumors. In adenomas, morphologic architecture is variable simulating *zona glomerulosa* or *zona fasciculata*. In tumors associated with Cushing syndrome, the adrenal cortex

surrounding the tumor is atrophic due to rebound pituitary *a*drenal *c*orticotropic *h*ormone (ACTH) suppression (Mc Nicol 2011). Carcinomas show lobulation with fibrous bands and areas of hemorrhage and necrosis.

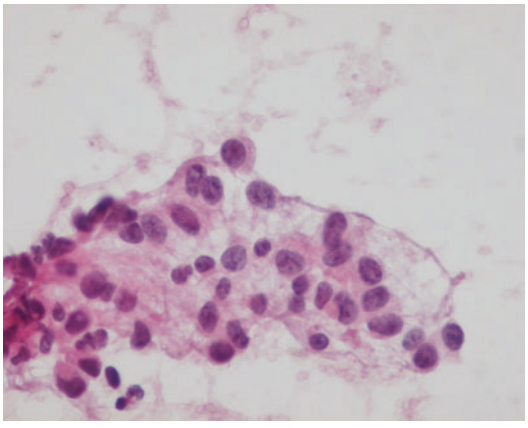
Fine-needle aspiration is not frequently required in the diagnosis of symptomatic adrenal nodules. Most diagnoses are performed based on the clinical set and image information. Surgery is frequently advanced without previous cytological diagnosis. Like in most endocrine tumors, cytological spectrum is wide, and criteria to differentiate nodular hyperplasia from adenomas and adenomas from carcinomas are poorly attempted in cytology. Fine-needle interpretation should be done with complete knowledge of clinical, radiologic, and biochemical findings (Hoang et al. 2002). These tumors can have a very pleomorphic or extremely bland appearance (Fig. 1), without that indicates the degree of aggressiveness. Mitotic count and vascular or capsular invasion seem to correlate with behavior, but these criteria are not applicable in cytological smears.

Most carcinomas are larger than 5 cm, and allied to this fact some authors found that in cytological smears, the presence of numerous mitosis, discohesive neoplastic cells, predominance of eosinophilic granular cytoplasm, marked nuclei pleomorphism, and prominent nucleoli (Figs. 2, 3) in a necrotic background were essentially present in carcinomas (Ren et al. 2006). Caution should be taken as carcinomas can be small and carcinomas can have cytological bland features.

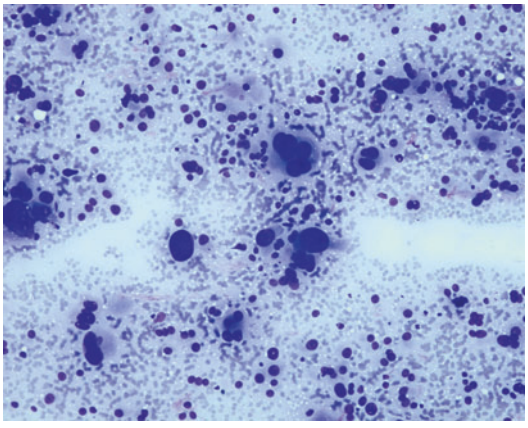
Smears in cortical adrenal adenoma and carcinoma are hypercellular, although cellularity is more remarkable in malignant lesions. Neoplastic cells lie in a lipidic background (only appreciated in Giemsa stains). Abundant nude nuclei lie single or overlapping in a morular ball pattern. Large tumor cells lie single or in poorly cohesive clusters. Tumor cells are polygonal or plasmacytoid with large eosinophilic or clear microvacuolated (more frequent in benign lesions) cytoplasm. Granular



Adrenocortical Tumors, Cytological Findings, Fig. 2 Adrenal cortical carcinoma with evident pleomorphism. Neoplastic cells are uni-, bi-, or multinucleated and nuclei are eccentric with pseudoinclusions (H and E 400x)



Adrenocortical Tumors, Cytological Findings, Fig. 1 Adrenal cortical adenoma with bland cytology (H and E 400x)



Adrenocortical Tumors, Cytological Findings, Fig. 3 Adrenal cortical carcinoma (Giemsa 400x)

eosinophilic and poorly defined cytoplasm are more frequent in carcinomas. Pleomorphism can be exuberant, and cells can have one or multiple atypical nuclei, mainly in malignant lesions; nucleoli can be prominent in these tumors, and nuclear pseudoinclusions or intracytoplasmic hyaline globules can be present. These aspects although not specific are more frequent in carcinomas. Necrotic background is generally seen in smears of carcinomas and is generally absent in adenomas. In some malignant tumors, cytology can be monotonous and even bland suggesting a benign lesion. In others neoplastic spindle cells can lead to a sarcomatous appearance, raising problems in the differential diagnosis with melanomas or pheochromocytomas.

Immunophenotype

Adrenal cortical tumors are immunoreactive for vimentin, alpha-inhibin, anti-Melan A antibody, cytokeratin CAM 5.2, neuron-specific enolase (NSE), calretinin, Hep Par1, and S100 protein (Pan et al. 2005). Immunoreaction with carcinoembryonic antigen (CEA) is absent; some tumors also stain for synaptophysin but are negative for chromogranin. These tumors are also negative for **H**uman **M**elanoma **B**lack (HMB45), **C**luster **D**esignation CD10, **C**ytokeratin CK7 and CK20, and **E**pithelial **M**embrane **A**ntigen (EMA) (Pan et al. 2005).

Besides helping in the correct diagnosis of adrenal cortical tumors versus other similar entities, immunostains can be helpful in predicting tumoral outcome.

P53 mutation/overexpression, high Ki67 proliferation index, *I*nsulin *G*rowth *F*actor IGF 2 overexpression, cyclin E, and beta-catenin can be helpful in establishing malignancy and predicting outcome (Soon et al. 2009).

Molecular Features

Adrenal cortical tumors can be sporadic or associated with syndromes like Li-Fraumeni, Beckwith-Wiedemann, familial adenomatous polyposis, and MEN1 syndrome.

Molecular studies become relevant in doubtful cases that do not fulfil all the criteria for unequivocal diagnosis as carcinoma. P53 mutation/overexpression, high Ki67 proliferation index, IGF 2 overexpression, cyclin E, and beta-catenin can be helpful in establishing malignancy (Soon et al. 2009).

Alterations in IGF 2 proteins and Ki67 proteins have been recently indicated as two parameters of diagnostic importance (Soon et al. 2009; Sasano et al. 2001). Mutations in the CTNNB1 gene in about 1/3 of adrenal carcinomas have also led to the contemplation that the Wnt pathway might also be involved in a small percentage of these tumors, (Tissier et al. 2005). In recent studies mutation on exon 3 of beta-catenin gene has been associated with high-grade carcinomas and more aggressive course (Tissier et al. 2005).

Differential Diagnosis

Differential diagnosis between adrenal cortical adenoma and carcinoma in small bland morphological looking cytologic smears, are in most situations impossible.

Monotonous and bland lesions raise sometimes problems in differentiating from small round cell tumors. Some adrenal adenomas show oncocytic features raising problems with contaminant aspirated hepatocytes.

► Small-cell carcinomas are characterized by regular nuclei with "salt and pepper" chromatin, nuclear molding, and a visible although scarce rim of cytoplasm. These aspects should not be confounded with nude nuclei in adrenal cortical tumors that generally have nucleoli and more anisokaryosis. Small-cell carcinomas are generally chromogranin and TTF1 positive, whether adrenal cortical tumors or not.

Oncocytic cortical adrenal adenoma or carcinoma poses differential diagnostic problems with renal oncocytic tumors, chromophobe renal carcinoma, angiomyolipoma, and even papillary carcinoma type II (Hoang et al. 2002). Differential diagnosis between these entities is rarely raised as this oncocytic pattern is also unusual. This

theme is discussed elsewhere as well as the characteristics of these tumors.

Adrenal gland due to its vascularization is a common place for metastasis. Renal cell carcinoma and hepatocarcinoma frequently metastasize to the adrenal gland.

Because of its wide morphologic spectrum, adrenal cortical tumors mainly carcinomas are frequently misdiagnosed as renal carcinomas (RCC), hepatocarcinomas (Pan et al. 2005), melanomas, and ▶ pheochromocytomas. Differentiating renal cell carcinoma from adrenal cortical tumors can be challenging, and immunostains are essential to accomplish this task. Renal cell carcinomas should be suspected in bloody smears with glycogen leakage (tigroid background seen in Giemsa stains). Neoplastic cells in RCC have small glycogen vacuoles in cytoplasm and coexpress cytokeratin and vimentin as well as EMA and CD10. Synaptophysin, Melan A, and alpha-inhibin are negative in RCC.

Differential diagnosis with hepatic carcinoma is more problematic, as adrenal cortical carcinomas can be positive for Hep Par1; however, hepatocarcinomas are negative for synaptophysin and alpha-inhibin.

Pheochromocytomas also share morphologic characteristics with adrenal cortical tumors; both lesions can be positive for synaptophysin, but positivity for chromogranin is only seen in pheochromocytomas.

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Anaplastic Carcinoma of Thyroid, Cytological Findings

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Synonyms

Giant and spindle cell carcinoma; Undifferentiated carcinoma

Definition

A rare primary carcinoma of the thyroid gland but one of the most aggressive malignant tumors.

Clinical Features

- **Incidence**
  It represents less than 5% of malignant tumors of the thyroid.
- **Age**
  Usually seen in people older than 50. Rarely seen before.
- **Sex**
  More often observed in women (sex ratio F/M 2–4:1).
- **Site**
  The tumor attacks very rapidly the thyroid gland in total with extension to the upper part of respiratory tract, soft tissues of the neck, bones, etc. Metastases are frequent, mostly lymph nodes and lungs metastases.
- **Treatment**
  Surgery, radiotherapy, and chemotherapy are three options; surgical treatment is chosen when the tumor is still more or less limited to the thyroid gland. A combination of surgery followed by radiotherapy has also been proposed. Recently, gene therapy combined with radioiodine has been attempted.
- **Outcome**
  Death in the next 3–6 months is the usual outcome.

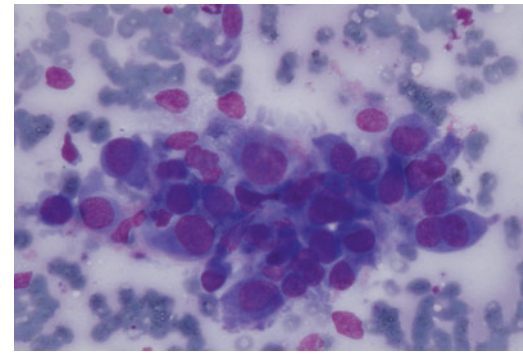
Macroscopy

The thyroid gland appears widely invaded by a tumor which is a white-tan colored and modified with large hemorrhagic and necrotic areas.

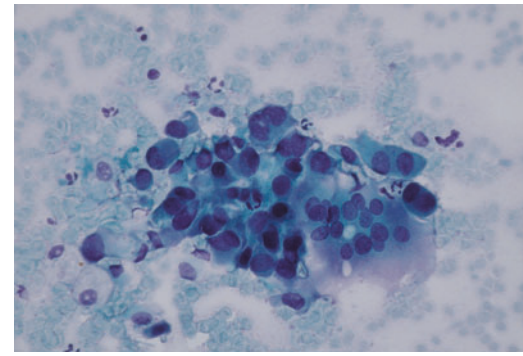
Microscopy

Fine-needle aspirations (FNA) are highly cellular and obviously malignant. There are epithelial cells of variable size, isolated or in groups, with round or polygonal or spindle cytoplasm. The nuclei are enlarged, irregular, pleomorphic, including huge nucleoli (Figs. 1 and 2). Some tumor cells are multinucleated cells; mitoses may be frequent. Necrosis is usually seen in the background without colloid, but with inflammatory cells like neutrophils, histiocytes, and some osteoclast-like giant cells. No specific arrangements such as papillary features or microfollicles are visible. In some cases, the FNA may be poorly cellular due to a marked fibrosis and then the diagnosis may be quite challenging.

Cytological criteria are similar on conventional smears and in liquid-based cytology.



Anaplastic Carcinoma of Thyroid, Cytological Findings, Fig. 1 Large sheet of more or less well-preserved cells showing marked anisokaryosis (conventional slide; MGG staining ×40)



Anaplastic Carcinoma of Thyroid, Cytological Findings, Fig. 2 Similar features (conventional slide; Papanicolaou staining ×40)

Immunophenotype

Anaplastic carcinomas are mostly pan-cytokeratin- and vimentin-positive. A positive thyroglobulin is very helpful to confirm the thyroid origin; TTF1 is often negative and less discriminant for the origin than thyroglobulin.

Molecular Features

Deregulation of the p53 pathway is an important step leading to the progression of well-differentiated carcinoma to poorly differentiated and undifferentiated carcinoma. In anaplastic carcinoma, some studies have also highlighted the role of the Wnt pathway, implying mutations in the β-*catenin* and *Axin1* genes, and of the PI3K/Akt pathway, through a mutation in PI3KCA.

Differential Diagnosis

They are many differential diagnoses:

In cases of sparse cellularity with inflammatory cells, there is a risk of a false-negative with a diagnosis of acute thyroiditis; both lesions may be painful and lead to a rapid enlargement of the gland. On FNA, very atypical reactive cells are sometimes not very different from true neoplastic cells; the context, the age, the clinical data and ultrasonography are helpful; a second FNA can be realized in order to obtain more representative cellular material.

In cases of medium or high cellularity, the differential diagnoses are: (1) a large cell lymphoma in which there are only isolated cells, usually more monotonous, without very atypical multinucleated tumoral cells; (2) a medullary carcinoma, poorly differentiated, with large neoplastic cells sometimes bi- or multinucleated; it is necessary to look carefully, in the background, for small plasmacytoid cells more typical for the diagnosis of a medullary carcinoma as well as for amyloid deposits; (3) metastasis are sometimes really difficult to distinguish morphologically from a primary thyroid anaplastic carcinoma especially when the primary tumor is a large cell lung carcinoma or a poorly differentiated squamous cell carcinoma; melanoma may also mimic anaplastic carcinoma of the thyroid when they show mixed epithelial and spindle neoplastic components without melanin pigment. For all these tumors, immunocytochemistry is necessary including lymphoid markers for lymphoma, chromogranin A and calcitonin for medullary carcinoma, HMB45 and S100 for melanoma, and cytokeratin 5/6 for squamous cell carcinoma; to exclude a large cell lung carcinoma thyroglobulin for a thyroid origin is the only helpful marker and may remain negative as we said.

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Angiomyolipoma, Cytological Findings

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Synonyms

Hamartoma

Definition

Angiomyolipoma (AML) is actually a choristoma which means that it is formed by the faulty development of a tissue type not normally found at that location (smooth muscle and fat). Angiomyolipoma is a mesenchymal benign tumor classically composed of blood vessels, smooth muscle cells, and fat cells. This tumor seems to

arise from epithelioid cells that surround blood vessels, with the capacity of adipose and smooth muscle differentiation. These cells are the origin of a wider group of tumors also called as PEComas (initials of perivascular epithelioid cell) and include lesions like pulmonary lymphangioleiomyomatosis, clear-cell sugar tumor, or clear-cell myomelanocytic tumor of the falciform ligament. This perivascular cell has not a normal counterpart, and so it is impossible to detect in normal tissue.

Clinical Features

- **Incidence**
  Angiomyolipoma has an incidence of about 0.3–3% and represents approximately 1% of surgically removed renal tumors.
  Most cases are asymptomatic. Large lesions can manifest themselves as palpable abdominal masses, and they may cause hematuria or flank pain due to sudden growth with massive hemorrhage. Clinical presentation can occur in two patterns: (1) sporadic (80% of the cases) or (2) in the context of tuberous sclerosis (TS) in 20% of the cases.
  On computed tomography (CT) scanning studies, the occurrence of fatty attenuation in a renal tumor is diagnostic.
- **Age**
  Eighty percent of patients with TS develop an AML. In this clinical setting they occur earlier in adolescence. In sporadic cases they tend to occur in older patients with a mean age of 43 years.
- **Sex**
  Both in sporadically and TS associate forms, genders are affected equally. However, there is a slight female predominance, in the context of tuberous sclerosis (Eble et al. 2004).
- **Site**
  Most angiomyolipomas arise in the kidney. Curiously, there seems to be a predominance of the right kidney.
  Cases ocuring in liver, parotid gland, upper lip, colon, and jejuna beyond others have been described (Tsui et al. 1999; Andaleeb et al. 2010; Silva et al. 2007; Abdulkader et al. 2005; Foschini 1999).
- **Treatment**
  AML treatment is conservative, particularly if they are asymptomatic. Annual follow-up is recommended for lesions smaller than 4 cm. In patients with hemorrhagic complications, the tumor should be resected. In patients with tuberous sclerosis, multiplicity and bilateralism is common, so whenever excision is demanded nephron conservation is of greater importance.
- **Outcome**
  Most angiomyolipomas are benign tumors.

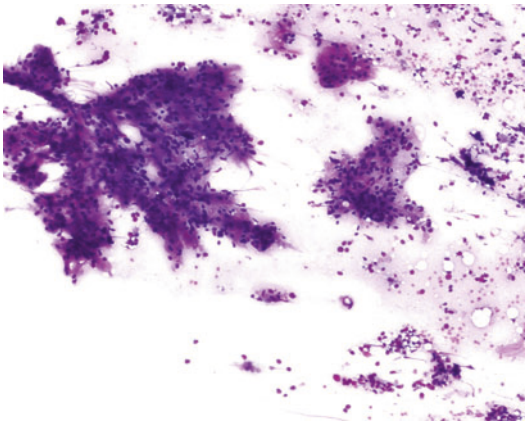
Macroscopy

AMLs are grossly round or lobulated and yellow to gray on cut sections according to their fat content. The tumor has expansible growth inside the kidney. There are some cases described originating from the capsule extension to the perinephric adipose tissue. In sporadic cases, AMLs are in most cases single, unilateral, and larger than those associated with TSC (Eble et al. 2004).

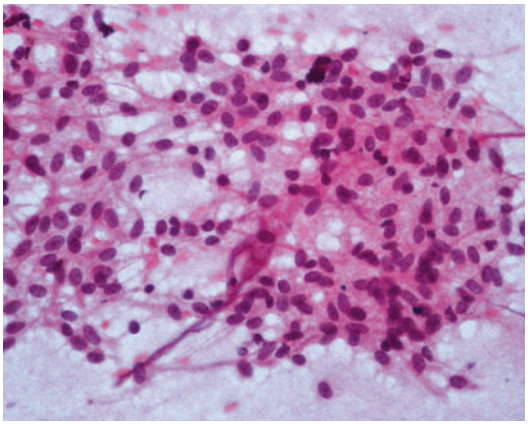
Microscopy

Most AML are uncapsulated but well-limited lesions. The majority, (88%), extend through the renal capsule into the perinephric space. AML is composed of various proportions of tortuous vessels (Fig. 1), smooth muscle, and fat (Fig. 2). The blood vessels frequently have an angiomatous arrangement with a disorganized adventitial cuff of smooth muscle but without an elastic tissue.

Angiomyolipomas displace the renal parenchyma and distort the collecting duct system. Different morphologic patterns can exist and coexist, in particular when associated to tuberous sclerosis:



Angiomyolipoma, Cytological Findings, Fig. 1 Angiomyolipoma in a tuberous sclerosis clinical setting. Scraped material. Remark the presence of tortuous vessels crossing the smear, seen in a background of lipidic droplets (Giemsa 400x)



Angiomyolipoma, Cytological Findings, Fig. 2 Angiomyolipoma in a tuberous sclerosis clinical setting. Material was scraped from the kidney tumor. Smears show a fragment of epithelioid cells (smooth muscle cells) (H&E 400x)

- *Classic angiomyolipoma* – tumor composed of variable amounts of adipose, smooth muscle, and thick-walled vessels.
- *Oncocytoma-like angiomyolipomas* – this pattern overlaps with that of oncocytoma. Coexistence of lesions, oncocytoma, and angiomyolipoma has been described in TS setting in literature (Theodosopoulos 2009). Immunohistochemistry is crucial in this differential diagnosis.
- *Epithelioid angiomyolipoma* – this variant is entirely composed of a proliferation of epithelioid cells, reactive to HMB45. No adipose or vascular components are present. Necrosis and atypia can be identified. These tumors can have an aggressive behavior and metastasize (Sood et al. 2007).
- *Cystic angiomyolipoma* – these tumors contain cysts lined by flat or cuboidal eosinophilic cells. Surrounding these cystic areas, a cambium layer separates it from the lipomatous or more frequently myomatous elements. This pattern must be distinguished from mixed epithelial-stromal neoplasms (Armah et al. 2007).
- *Microscopic angiomyolipomas* – small nodules often present in tuberous sclerosis kidneys with multiple angiomyolipomas. These are morphologically heterogeneous lesions generally without the typical thick-walled vessels.
- *Intraglomerular lesions* – intraglomerular clusters of epithelioid smooth muscle.

On cytology the diagnosis of classic AML should be considered whenever an admixture of blood vessels, fat, and smooth muscle cells is found.

The presence of adipose tissue in a fine-needle aspiration cytology of a renal mass should alert to this diagnosis. Typical thick-walled vessels identified in histology are hardly seen in smears but can be identified in cell-blocks. Smooth muscle cells have an epithelioid appearance. Occasional nuclear atypia is admitted.

In epithelioid variants a predominant population of epithelioid cells is seen. The cells are generally disposed in islands or small aggregates and have a low N:C ratio with central round nuclei and fine chromatin. Some authors also refer the presence of nuclear pseudoinclusions (Armah et al. 2007; Ingle et al. 2010). These cells have fragile cytoplasm, and it is not infrequent to see nude nuclei. Whenever the cytoplasm is perceptive, it varies from fine vacuolated to dense cyanophilic. In this epithelioid category, the presence of smooth muscle and adipose component can be very scarce (Armah et al. 2007).

Angiomyolipoma, Cytological Findings, Table 1 Kidney eosinophilic tumors – immunophenotype

	Chromophobe RCC (eosinophilic)	Clear-cell RCC (eosinophilic)	Oncocytoma	C. papillary (type II)	Angiomyolipoma
Colloidal iron	+ ++	–	–	–	–
Racemase	–	–	–	+++	–
Vimentin	–	++ +	–	++ +	+
CD10	–	++	–	++	–
EMA	+	++	+		–
CK7	+	–	–	+Focal in type II	–
CAM 5.2	+	+	+		–
Cadherin E	+	–	+	+ (40% of type II)	
CD117	+++	–	+++	–	+
Parvalbumin	+++	– (++ in sarcomatoid)	+++	–	
RCC	–	+++	–	++	
HMB45	–	–	–	–	+++
Melan A	–	–	–	–	+++

Immunophenotype

AML express myogenic and melanocytic markers, such as *H*uman *M*elanoma *B*lack (HMB45), *H*uman *M*elanoma *S*tain *A*ntigen (HMSA-1), Melan A/melanoma-associated antigen recognized by T cells (Mart1), microphthalmia transcription factor (Mitf), smooth muscle actin, and, less commonly, desmin.

Molecular Features

Both inherited and sporadic AML frequently demonstrate loss of heterozygosity of chromosome 16p (containing the TSC2 locus). The TSC1 gene occasionally shows loss of heterozygosity.

Differential Diagnosis

On cytology differential diagnosis arises when one misinterprets the adipose and vascular tissues as local perirenal tissue. In these cases, the only element that is regarded as the neoplastic is the smooth muscle component. This observation error can lead to suspect the existence of a sarcoma. AML with epithelioid cells or with eosinophilic predominant cells pose other problems. Differential diagnosis with eosinophilic variants of epithelial renal tumors should be raised. Included are oncocytoma, renal cell carcinoma (eosinophilic variant), chromophobe carcinoma, and even papillary carcinoma type II. This issue is discussed with renal cell carcinoma, chromophobe carcinoma, and oncocytoma. In these renal tumors with overlapping features, immunostains and sometimes molecular studies are crucial in the differential diagnosis (Table 1).

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Apocrine Carcinoma, Cytological Findings

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Synonyms

Invasive apocrine carcinoma

Definition

The term apocrine carcinoma should be used for neoplasms in which all or nearly all the epithelium shows apocrine cytological features. In contrast, focal apocrine differentiation is quite common and has been reported in up to 60% of carcinomas of no special type (NST).

Clinical Features

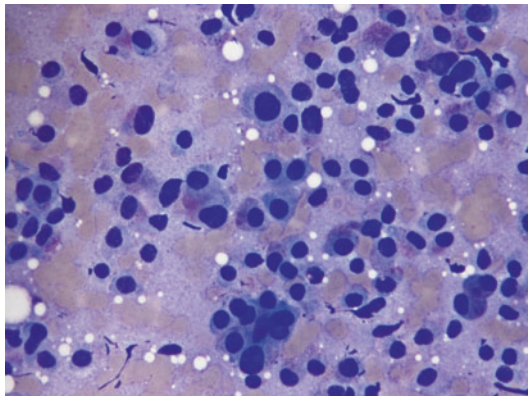
- **Incidence**
  The incidence of pure apocrine carcinoma varies from <0.3% to 4%.
- **Age**
  Similar to the ductal invasive carcinoma.
- **Sex**
  Predominance in women.
- **Site**
  Few reports that specify the laterality of the disease show the predominance of the left side, a fact that should be interpreted with caution.
- **Treatment**
  Surgery, radiotherapy, chemotherapy, and hormonotherapy.
- **Outcome**
  No statistical difference in estimated recurrence-free survival or overall survival with breast carcinomas without apocrine differentiation.

Macroscopy

Grossly, these tumors are indistinguishable from other mammary carcinomas.

Microscopy

Cytological features were characterized by numerous cells arranged singly or arranged in syncytial fragments. The cells presented large nuclei with prominent nucleoli and abundant eosinophilic and granular cytoplasm. The cell outline is polygonal.



Apocrine Carcinoma, Cytological Findings, Fig. 1 Apocrine carcinoma. Note the nuclei atypia and granular cytoplasm (Giemsa staining)

Histologically, apocrine carcinomas show the same architectural growth pattern as other NST mammary carcinomas, differing only in their cytological appearance. It is defined by large cells with abundant eosinophilic cytoplasm, usually granular, in cells with a nucleus/cytoplasm ratio of 1:2 or more (Fig. 1).

Immunophenotype

Apocrine cells show immunoreactivity for GCDFP-15, epithelial membrane antigen, and cytokeratins 8 and 18, but these are not specific for the apocrine cell type. Alpha-1 antitrypsin and lysozyme are occasionally positive. Characteristically, the cells are also known to be BCL2 and S100 negative. ER and PR are also negative while AR is consistently positive. HER2 can be positive.

Molecular Features

These include losses at 2p and 9q and gains at 2q, 3p, and 13q.AR-positive and ER-negative hormone profiles and HER2 amplification. An "apocrine molecular signature" identified by gene-expression array analysis is characterized by increased androgen signaling and overlap with HER2 overexpressing group.

Differential Diagnosis

Apocrine cells are often seen in association with macrophages and benign duct epithelial cells in smears from fibrocystic change and also in smears from breast cyst fluids. Benign apocrine cells may occasionally be rather worrisome in breast cysts, especially if these are inflamed. Atypical apocrine cells from fibrocystic change, and especially apocrine change in sclerosing adenosis, may be extremely difficult to distinguish from apocrine carcinoma. If there are mitoses or if necrosis is present, carcinoma is the most likely diagnosis; but if these are absent, it is wise to be cautious.

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Astrocytoma, Cytological Findings

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Synonyms

Anaplastic astrocytoma (Astrocytoma grade 3); Diffuse astrocytoma; Glioblastoma (Astrocytoma grade 4)

Definition

Neoplasms composed by astrocytes with variable differentiation and infiltration with a spectrum of astrocytoma, anaplastic astrocytoma, and glioblastoma.

Clinical Features

- **Incidence**
  Astrocytoma represents 10–15% of astrocytic neoplasias and glioblastoma 60–75% of astrocytic tumors.
- **Age**
  The age distribution of astrocytoma shows a peak incidence between 30 and 40, anaplastic astrocytoma around 40 years, and glioblastoma affects adults between 45 and 75 years.
- **Sex**
  There is a predominance of male in astrocytomas and glioblastoma.
- **Site**
  Mainly on cerebral hemispheres.
- **Treatment**
  The care of patients with astrocytomas in different grades involves surgery, radiotherapy, and chemotherapy.
- **Outcome**
  The mean survival in astrocytomas is in the range of 6–8 years and is influenced by the progression to glioblastoma. Anaplastic astrocytoma has a strong tendency to progress to glioblastoma a mean interval of 2 years. The overall survival of patients with glioblastoma is poor and less of 20% survival than 1 year.

Macroscopy

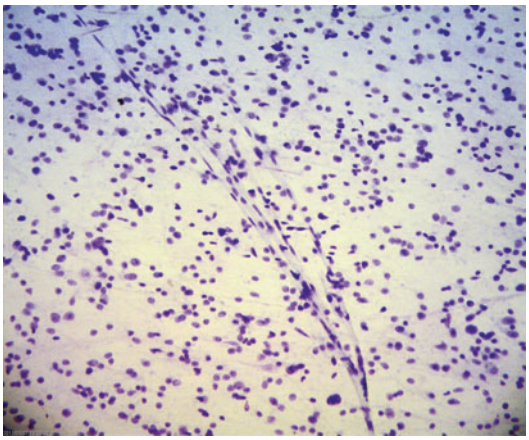
Astrocytoma shows local mass with indistinct boundaries, cystic changes are common. It has homogeneous appearance, and tumor margin is usually not identifiable.

Glioblastoma is a firm grayish-brown tissue with areas of hemorrhage and necrosis.

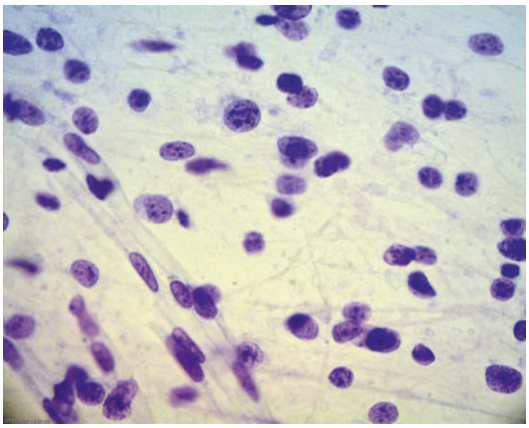
Anaplastic astrocytoma has the same appearance of glioblastoma without hemorrhage and necrosis.

Microscopy

Astrocytoma presents moderately cellularity with some degree of pleomorphism, vesicular nuclei with clumped chromatin, and a prominent fibrillary and metachromatic background and delicate vessels (Figs. 1 and 2).



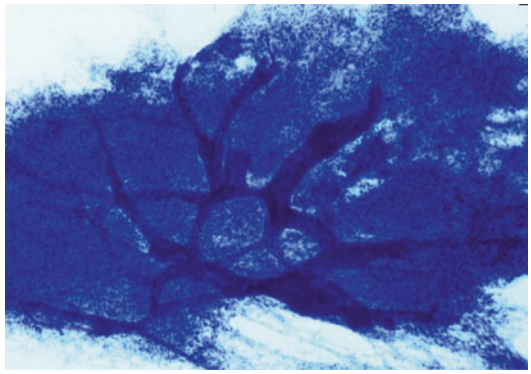
Astrocytoma, Cytological Findings, Fig. 1 Astrocytoma – smear with astrocytic homogeneous population and delicate vessels (Toluidine blue)



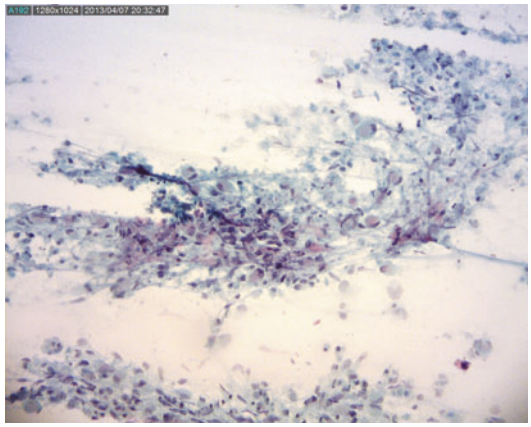
Astrocytoma, Cytological Findings, Fig. 2 Astrocytoma – Note pure astrocytic proliferation and fibrillar matrix. Astrocytes with minimal pleomorphism, kidney-shape nuclei, and chromatin dotted. Absence of mitosis or vessels proliferated (Toluidine blue)

Glioblastoma presents very cellular smears with anaplastic neoplastic cells closely related to blood vessels, with prominent vascular endothelial proliferation (Fig. 3). Numerous mitoses and evidence of necrotic tissue or scattered neutrophils are seen (Fig. 4).

Anaplastic astrocytoma appear similar to glioblastoma except that no necrotic and scattered neutrophils are seen and residual fibrillar background (Fig. 5).



Astrocytoma, Cytological Findings, Fig. 3 Glioblastoma – Note high cellularity and large anomalous vessels proliferated (Toluidine blue)



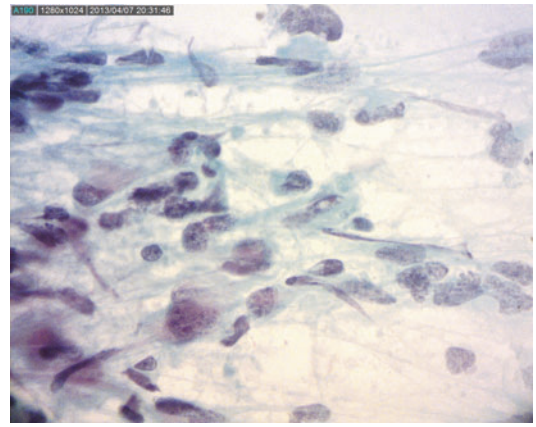
Astrocytoma, Cytological Findings, Fig. 4 Glioblastoma – Note astrocytes with severe atypia and necrotic debris (Papanicolaou)

Immunophenotype

Glial fibrillar acid protein (GFAP) is expressed in a variable degree. Activity mitotic growth fraction maybe determined in Anaplastic Astrocytoma by Ki-67/MIB-1.

Molecular Features

Astrocytomas and anaplastic astrocytomas present mutations of TP53 and loss of heterozygosity of LOH 10p. These changes in glioblastoma are added by EGFR amplification.



Astrocytoma, Cytological Findings, Fig. 5 Anaplastic astrocytoma – Note atypical astrocytes and residual fibrillar background (Papanicolaou) (Note: Source of the figures of the authors)

Differential Diagnosis

Astrocytoma – differential diagnosis include reactive proliferation of astrocytes, others gliomas like anaplastic astrocytoma, glioblastoma, oligodendroglioma, and ependymoma.

Anaplastic Astrocytoma – the differential diagnosis are reactive astrocytic process and gliobastoma.

Glioblastoma – differential diagnosis includes abscess and metastasis of carcinomas and melanomas. In many cases of lymphoma, there are a florid reactive gliosis which may be mistaken for glioblastoma.

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Atypia, Chemotherapy-Associated

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Synonyms

Chemotherapy-associated atypia; Cytotoxic drugs–associated atypia

Definition

Chemotherapy and radiotherapy are used for the treatment of cervical cancer. Chemotherapy-associated atypia is the effect of cytotoxic drugs on the cervical epithelium leading to cytologic atypia, which can lead to an erroneous diagnosis of preneoplastic dysplasia in cervical smears. This effect varies according to dosage, period of exposure, and the intervals between treatments (Titmuss and Adams 2007). The effects can last for several years (Titmuss and Adams 2007). Cyclophosphamide is the drug most strongly associated with epithelial dysplasia (Hughes et al. 1993; Westra et al. 2001) administered either alone (Brien et al. 2001; Ognenovski et al. 2004) or with busulfan (Castano et al. 2002; Stella et al. 1990).

Microscopy

The cytologic features include bizarre atypia with marked cellular enlargement exceeding that seen in cancer (macrocytosis/"ballon-shaped" cells), lower nuclear to cytoplasmic ratio, irregular nuclear membrane, cytoplasmic polychromasia and vacuolation, lower mitoses, atypia also involving fibroblasts and endothelial cells (Bateman et al. 2000; Walker et al. 2002). Prominent, multiple, or eosinophilic nucleoli (Westra et al. 2001; Slavin et al. 1975) and smudging of the chromatin (Belinson et al. 1985) are common in chemotherapy-related atypia. Multinucleation as well as leucophagocytosis may be noted.

Immunophenotyping

Ki-67 proliferation index is low in these lesions (Westra et al. 2001).

Differential Diagnosis

Chemotherapy-associated a type should be differentiated from high-grade dyskaryosis and invasive carcinoma.

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Atypical Glandular Cells

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Synonyms

AGC

Definition

Atypical glandular cells (AGC) should be qualified as endocervical or endometrial in origin, whenever possible. If not, a generic diagnosis of "atypical glandular cells not otherwise specified" (NOS) is appropriate. Atypical endocervical cells (AEC) are defined as endocervical glandular cells with morphologic features that are beyond reactive/reparative changes but are yet unequivocal for a diagnosis of endocervical adenocarcinoma in situ (AIS) or endocervical adenocarcinoma. These may be further categorized as "favor neoplastic" if the morphologic features of atypia are more pronounced and more in favor of a neoplastic process but still fall short those of AIS or adenocarcinoma. When criteria are qualitatively and quantitatively sufficient, a diagnosis of AIS can be made. AIS is the precursor to invasive endocervical adenocarcinoma. Atypical endometrial cells (AEM) are endometrial glandular cells that display atypical features beyond those of benign-appearing endometrial cells. The atypia of endometrial cells could be due to neoplastic or nonneoplastic conditions that may not be reliably distinguished from each other. Hence, there is no category of "favor neoplastic" for further classification of atypical endometrial cells.

Clinical Features

- **Incidence**
  The diagnosis of AGC accounts for a minor fraction of Pap test results, ranging from 0.1 to 0.6% in most studies. As per the College of American Pathologists (CAP), the reporting rates for AGC for the laboratories participating in their Interlaboratory Comparison Program ranges from 0.0% to 1.2%. The mean reporting rate for all specimen types combined is reported to be 0.39% with the 50th percentile at 0.2%.
  See also entry on "► Endocervical Adenocarcinoma, Invasive"
- **Age**
  The diagnosis of AGC is seen in a wide age range (16–96 years) with a mean of 44 years. The mean age for diagnosis of AIS is 37 years. Women with AEM tend to be older than those in other categories with a mean of 50 years.
- **Site**
  The atypical glandular cells seen in a Pap test can originate from different body sites –

transformation zone and endocervix (most commonly), lower uterine segment, uterine corpus, occasionally adnexa (fallopian tubes and ovaries), and rarely extragenital sites such as colon.

- **Treatment**
  As per the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines, all women with AGC subcategories except AEM should undergo colposcopy with endocervical sampling. In women who are 35 years of age or older or those who are clinically at risk for endometrial neoplasia, endometrial sampling is recommended in addition to colposcopy and endocervical sampling. For women with AEM, the preferred management is endometrial and endocervical sampling at initial evaluation. If no endometrial pathology is identified, then colposcopy is recommended. It is important to note that these management guidelines stand regardless of the HPV status of the woman. Triage by reflex HPV testing is not recommended for AGC cases as endometrial adenocarcinomas and some endocervical adenocarcinomas (i.e., the mucinous type) are not related to HPV infection.

  In terms of subsequent management, if no high-grade squamous intraepithelial lesion or glandular neoplasia or cancer is identified after a diagnosis of AGC-NOS or AEC or AEM, co-testing is recommended at 12 and 24 months. If both co-tests are negative, then co-testing can be repeated in 3 years. If any of the tests is abnormal, colposcopy is recommended. If the Pap test diagnosis was AIS or AGC- favor neoplastic and no invasive disease is detected on biopsy then a diagnostic excisional procedure is recommended. For pregnant women, the management is same except that endocervical curettage and endometrial sampling should not be performed.

  The definitive management for AIS is hysterectomy. However, if fertility is desired, then an excisional procedure (cold knife cone biopsy or LEEP) with negative margins can be performed with continued follow-up.

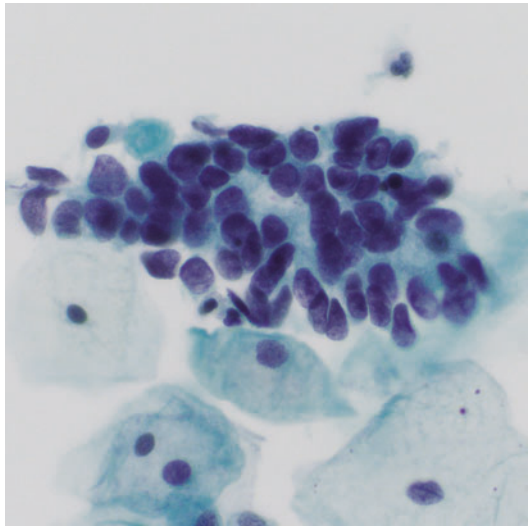
- **Outcome**
  The majority (66–70%) of AGC diagnoses are associated with a benign histologic outcome. However, there are a small proportion of cases that show significant precancerous or malignant outcomes. Most of these lesions are squamous dysplasias. Of the glandular abnormalities, endometrial neoplasia is more commonly seen than endocervical neoplasia. Approximately 5% of cases are associated with cancers of cervix, endometrium, and rarely of ovary/fallopian tube. In comparison to AGC, endocervical glandular neoplasia is found more frequently with the diagnosis of AEC, and endometrial hyperplasia and adenocarcinoma is more commonly associated with the diagnosis of AEM. If we stratify the significant outcomes by age, squamous neoplasia is more likely in women <40 years and endometrial neoplasia is more likely in women >50 years.

Macroscopy

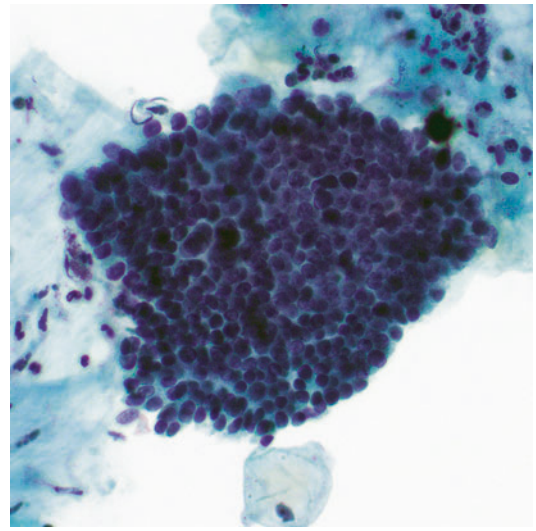
AEC and AIS are usually asymptomatic with a normal exam and minimal colposcopic findings. The lesion may be located high up in the endocervical canal, making it difficult to visualize. AEMs can be associated with abnormal vaginal bleeding and/or thickened endometrium on ultrasound, especially in postmenopausal women.

Microscopy

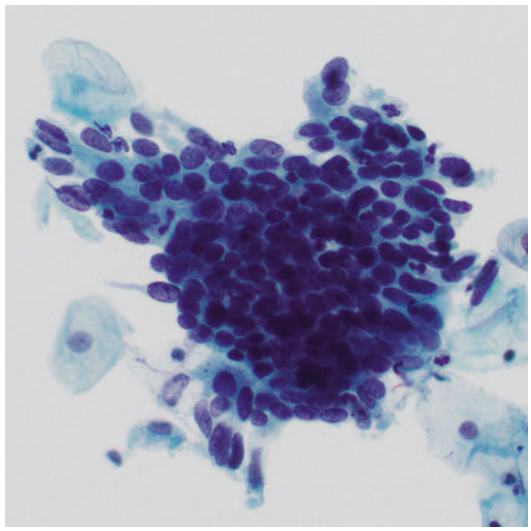
- **AEC, AEC-favor neoplastic and AIS**
  These subcategories are associated with a spectrum of glandular changes with AEC at lower end of the spectrum showing some of the features and AIS at the higher end depicting most if not all the features. Architecturally, the endocervical glandular cells occur in sheets and strips. There is mild to moderate nuclear crowding and pseudostratification. The cells may form rosette-like arrangements (Fig. 1). Also, the nuclei may protrude beyond the



Atypical Glandular Cells, Fig. 1 Atypical endocervical cells with hyperchromatic nuclei in rosette-like arrangement (ThinPrep, Papanicolaou stain, 400×)



Atypical Glandular Cells, Fig. 3 Monotonous appearing atypical endocervical cells with enlarged, crowded, and hyperchromatic nuclei and inconspicuous nucleoli (ThinPrep, Papanicolaou stain, 400×)



Atypical Glandular Cells, Fig. 2 Atypical endocervical cells with nuclear crowding and peripheral feathering (ThinPrep, Papanicolaou stain, 400×)

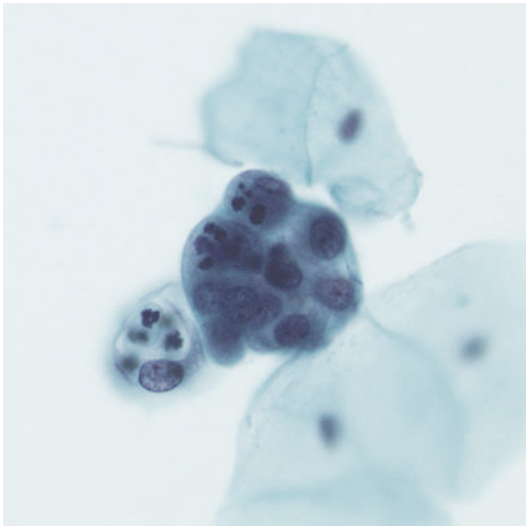
edge of a cluster – referred to as "feathering" (Fig. 2). The cells have a higher nuclear-cytoplasmic ratio as compared to normal endocervical cells. Nuclei are enlarged and elongated. The chromatin is coarsely granular and the nucleoli are inconspicuous. The nuclear atypia in these cells tends to be monotonous (Fig. 3). Mitotic figures and apoptotic bodies may be seen.

Occasionally, variants of AIS may be encountered that may be difficult to identify. Endometrioid AIS is characterized by smaller cells that show nuclear crowding and nuclear hyperchromasia. Focal feathering and occasional mitotic figures may be seen. Intestinal type of AIS shows nuclear crowding and overlap with prominent goblet-type cells.

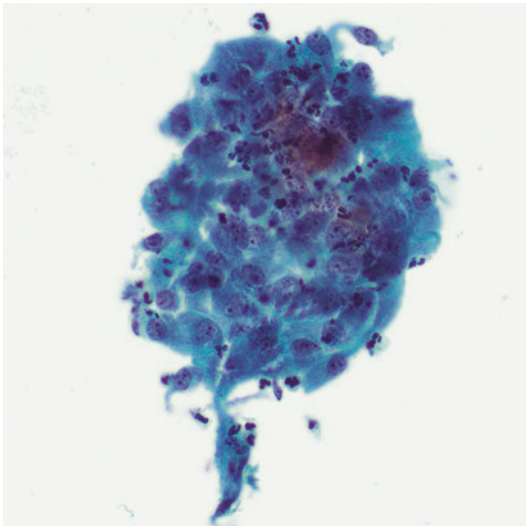
- **AEM**

  In contrast to benign-appearing endometrial cells, AEMs primarily show enlarged nuclei. The nucleus is enlarged in comparison to an intermediate cell nucleus. Other features that may be seen include small or prominent nucleoli, heterogeneous chromatin, and presence of intracytoplasmic neutrophils (Fig. 4).

  As mentioned previously, the diagnosis of AGC-NOS is used when the cells are atypical, but it cannot be determined if they are endocervical or endometrial in origin.



Atypical Glandular Cells, Fig. 4 Atypical endometrial cells with enlarged nuclei and intracytoplasmic neutrophils (ThinPrep, Papanicolaou stain, 400×)



Atypical Glandular Cells, Fig. 5 Reactive endocervical glandular cells in a flat arrangement with low nuclear-cytoplasmic ratio, finely distributed chromatin, and visible nucleoli (ThinPrep, Papanicolaou stain, 400×)

Immunophenotype

Cell blocks can be prepared from residual liquid-based cytology specimens that can assist in the distinction between AIS and its mimics such as tubal metaplasia. p16 immunohistochemical stain has been the most well studied in this regard. AIS is strongly and diffusely positive for p16 while tubal metaplasia usually shows a patchy staining pattern.

Molecular Features

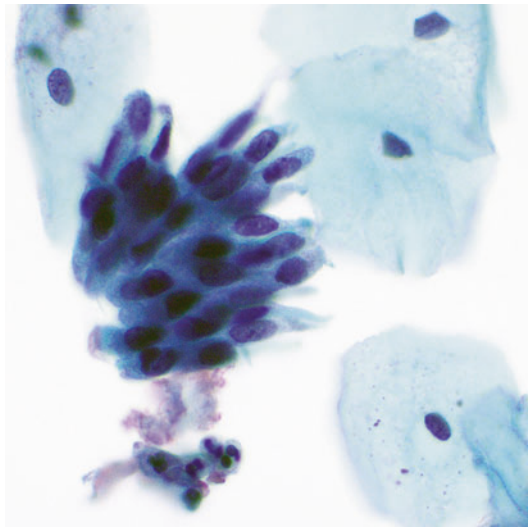
- **AIS**
  See entry on "▶ Endocervical Adenocarcinoma, Invasive"
- **AEM**
  AEM can be associated with endometrial hyperplasia or adenocarcinoma (usually endometrioid type) on histologic follow-up. Atypical endometrial hyperplasia and endometrioid adenocarcinoma show similar molecular genetic alterations including mutations in the PTEN tumor suppressor gene, mutations in the KRAS oncogene, and microsatellite instability.

Differential Diagnosis

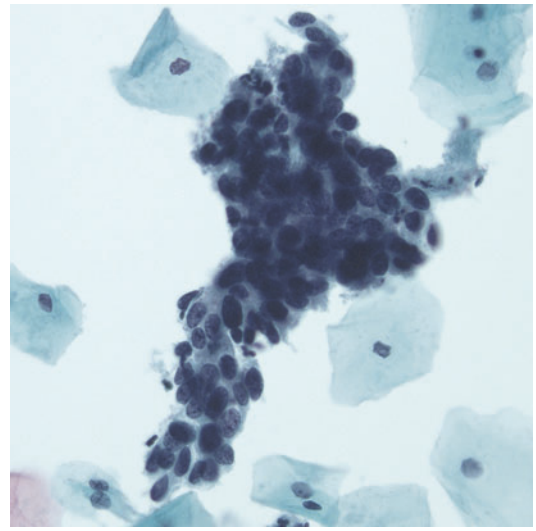
- **AEC, AEC-favor neoplastic and AIS**
  Reactive endocervical cells – Reactive endocervical cells typically have a flat architecture but may show mild nuclear crowding. The nuclei may be enlarged, but the nuclear-cytoplasmic ratio remains low. The chromatin is fine with visible to prominent nucleoli. Rosette formation and feathering are not typically seen. Mitoses may be seen but apoptotic bodies are not encountered. Similar findings may also be seen in endocervical polyps (Fig. 5).

  Tubal metaplasia – Tubal metaplasia can show pseudostratification and mild nuclear crowding. Feathering may be seen occasionally. There is mild nuclear hyperchromasia with indistinct nucleoli. The defining feature of tubal metaplasia is the presence of cilia and terminal bars (Fig. 6).

  High-grade squamous intraepithelial lesion (HSIL) – HSIL involving glands can appear similar to AIS. These groups usually show more nuclear crowding with a flattened edge,



Atypical Glandular Cells, Fig. 6 Tubal metaplasia – the cells exhibit pseudostratified and hyperchromatic nuclei. The defining feature is the presence of terminal bars and cilia (ThinPrep, Papanicolaou stain, 400×)



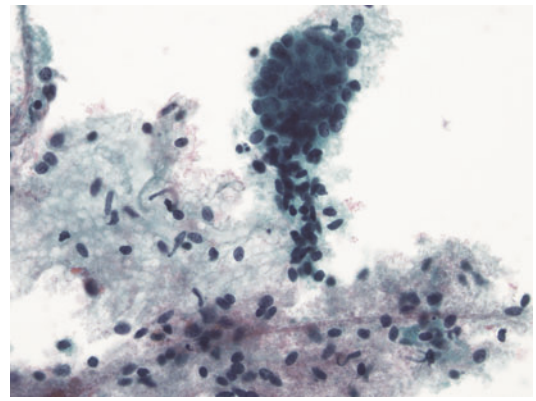
Atypical Glandular Cells, Fig. 7 High-grade squamous intraepithelial lesion with glandular involvement – the cell arrangement has a flattened edge, there is nuclear crowding, and the chromatin is coarsely granular (ThinPrep, Papanicolaou stain, 400×)

denser cytoplasm, and coarser chromatin distribution (Fig. 7).

Lower uterine segment endometrium (LUS) – Directly sampled LUS can be difficult to distinguish from AIS especially in patients who have undergone an excisional procedure such as LEEP, cone biopsy, or a trachelectomy. The endometrial cell fragments tend to be closely associated with stromal cells, but these two components may be separately seen in liquid-based preparations. The endometrial cells are arranged in sheets or branching tubules. They have a well-organized architecture with round to oval nuclei. The stromal cells have indistinct cytoplasm and bland nuclear chromatin (Fig. 8). The presence of stromal cells aids in the distinction of LUS from endometrioid AIS.

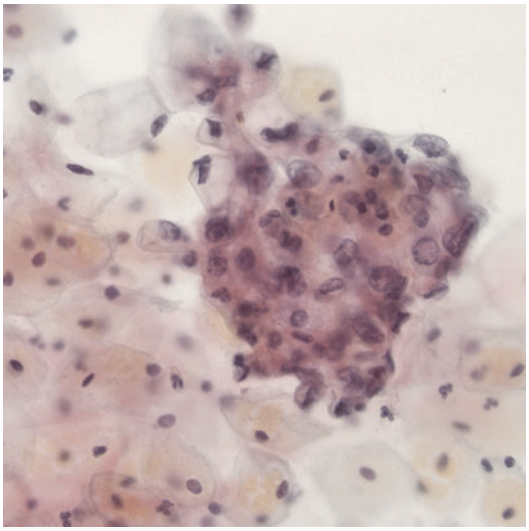
Endometriosis – See entry on "► Endometriosis, Cytological Findings"

Arias-Stella Reaction – Arias-Stella reaction in Pap tests can be seen in women who are pregnant or postpartum. Typically, there is

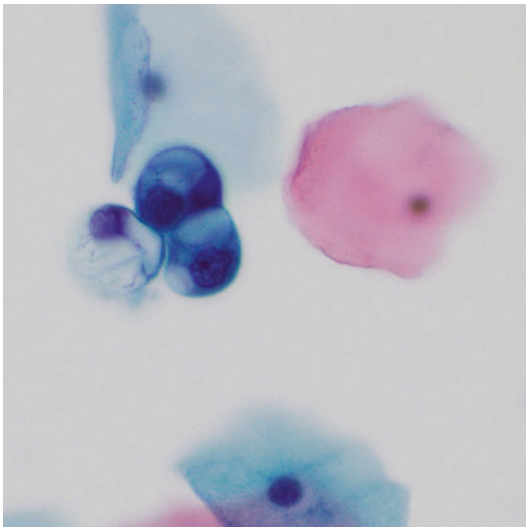


Atypical Glandular Cells, Fig. 8 Lower uterine segment endometrium – biphasic fragment composed of bland, uniform glandular cells closely associated with stromal cells (ThinPrep, Papanicolaou stain, 400×)

cellular and nuclear enlargement. The cytoplasm can be scant to abundant and can have vacuoles. The chromatin is granular or smudgy and intranuclear inclusions may be seen



Atypical Glandular Cells, Fig. 9 Arias-Stella reaction – cells have eosinophilic to clear cytoplasm, enlarged nuclei with abundant cytoplasm, and fine chromatin (Conventional smear, Papanicolaou stain, 400×)



Atypical Glandular Cells, Fig. 10 Cells associated with intrauterine contraceptive device (IUD cells) showing cytoplasmic vacuolation (ThinPrep, Papanicolaou stain, 400×)

(Fig. 9). The clinical information regarding the patient's pregnant/postpartum status is most helpful in distinguishing Arias-Stella reaction from AGC or malignancy.

- **AEM**

  Polyps – Endocervical polyps can show enlarged cells with vacuolated cytoplasm and engulfed neutrophils that can closely resemble AEM.

  Intrauterine device (IUD) – Patients with IUD can show vacuolated glandular cells in Pap tests that mimic AEM. The clinical history of IUD can help in their distinction from AEM (Fig. 10).

  Adenocarcinoma – Adenocarcinoma cells show more obvious architectural and cytologic abnormalities as compared to AEM. The cells are larger and could be seen singly or in groups with marked crowding. There can be marked nuclear pleomorphism with vesicular to coarse chromatin and prominent nucleoli. Also a background of necrosis (tumor diathesis) may be present.

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Atypical Immature Metaplasia

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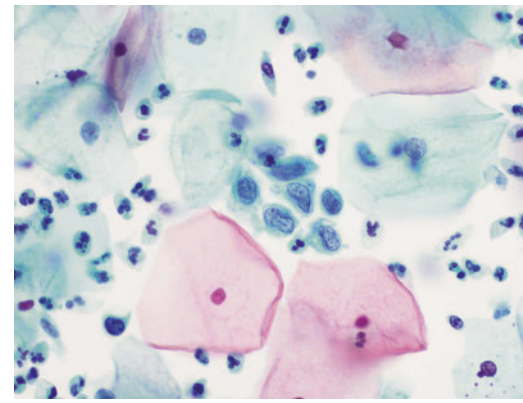
Synonyms

AIM; Atypical immature metaplasia; Atypical immature squamous metaplasia

A typical immature squamous metaplasia (AIM) refers to a full-thickness intraepithelial basaloid lesion in the uterine cervix that features both metaplasia and atypia and is therefore difficult to differentiate from high-grade cervical intraepithelial neoplasia (CIN III)/severe dyskaryosis. The biological and clinical significance of AIM is unclear. AIM was first described by Crum et al. (1983) in 1983 and then further characterized in 1999 as a lesion "which exceeds the limits of typical squamous metaplasia. Features typical of this group of epithelial alterations include immature squamous cells, which contain greater degrees of nuclear atypia or nuclear crowding, increased mitosis, and absence of normal differentiation, yet maintain the growth pattern of immature metaplastic epithelium" (Park et al. 1999). Based on nuclear crowding, nuclear atypia, and cytoplasmic maturation, AIM may be further subclassified into (1) "favor reactive," (2) not otherwise specified (NOS), and (3) "favor high-grade squamous intraepithelial lesion (HSIL)." The prevalence of human papilloma virus (HPV) DNA in these categories with consensus diagnoses of favor reactive, NOS, and favor HSIL was 7.7%, 28.6%, and 37.5%, respectively (Park et al. 1999; Iaconis et al. 2007). AIM represents a spectrum of lesions, from HPV-negative, benign, immature squamous metaplasia to high-risk HPV (hrHPV)-positive cases representing HSIL or HSIL precursor lesion. HPV testing (Geng et al. 1999), p16 immunostaining (Kong et al. 2007), and CINtec® Plus (dual immunostaining with p16 [a cyclin kinase inhibitor and a cell cycle regulatory protein] and Ki-67 [a proliferation marker]) may be of help to differentiate benign cases from potentially preneoplastic lesions (Schimdt et al. 2010). Cytokeratin (CK) 17 is a marker for cervical reserve/stem cells, which give rise to metaplasia. According to Regauer S et al., AIM was characterized by strong, uniform CK17 staining of the proliferating cells with concomitant p16 negativity, while high-grade dysplasias/severe dyskaryosis showed strong diffuse staining with p16 (Regauer and Reich 2007).

Cytomorphology

Immature metaplastic cells of the cervix resemble basal/parabasal cells. These may be present in sheets or as dispersed population (Fig. 1)



Atypical Immature Metaplasia, Fig. 1 A loose cluster of atypical immature metaplastic cells of the cervix with vesicular evenly distributed nuclear chromatin and moderate amount of well-defined cytoplasm (Pap ×100)

(Regauer and Reich 2007). Squamous or glandular differentiation may be evident depending upon the stage of maturation of the cells. The forceful detachment of these cells from the epithelium may lead to prominent cell angularity, which creates the characteristic "star" or "spider" shape (Titmuss and Adams 2007). In the unstable environment of the transformation zone, premature keratinization may occur imparting deep orangeophilia to the cytoplasm of these cells. Nuclei may vary in size, with vesicular chromatin and a high nuclear to cytoplasmic ratio (Titmuss and Adams 2007). Cytoplasmic vacuolization or presence of intracytoplasmic polymorphs may also be noted. Degenerative nuclear changes in the form of pyknosis, karyorrhexis, and karyolysis may be seen (Levine and Gray 2010).

Differential Diagnosis

Squamous dyskaryosis and glandular abnormalities.

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Atypical Squamous Cells of Undetermined Significance (ASC-US)

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Synonyms

Borderline changes in squamous cells; Borderline nuclear abnormalities

Definition

The term "atypical squamous cells" (ASC) is used by cytologists to describe equivocal morphological changes in squamous epithelial cells of the cervix. The term is specific to the Bethesda terminology, which is a widely recognized classification system for reporting cervical cytology (Solomon et al. 2002). The Bethesda System (TBS) stresses the importance of qualifying an ASC diagnosis into "atypical squamous cells of undetermined significance" (ASC-US) and "atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion" (ASC-H).

Morphological changes in cervical epithelial cells lie on a spectrum from the perfectly normal to definitely abnormal (neoplastic). The nature of cervical cytology is such that there will always be a proportion of cases for which doubt exists over the significance of morphological changes seen in one or more cells. The ASC-US report is therefore used to convey diagnostic uncertainty and should be considered as a holding category, in which definitive management is deferred until a repeat test or colposcopy can be arranged. In contrast to the more robust cytological changes associated with neoplastic disease, there is no reliable histological or clinical counterpart to ASC-US. Studies reporting on clinicopathological outcomes have shown that ASC-US represents a wide spectrum of entities, from minor inflammatory processes to invasive cancer. Although estimates vary considerably, up to 65% of ASC-US cases may be associated with a ▶ squamous intraepithelial lesion (SIL). In many cases, however, no abnormality is found on repeat testing and one must assume that the use of this reporting category represents a cautious approach to cervical cytology reporting. The concept of ASC-US therefore derives from the subjective visual interpretation of cervical preparations and is generally regarded as an unfortunate side effect of cervical screening.

Clinical Features

- **Incidence**
  As the majority of ASC-US reports represent an artifact of cervical screening rather than genuine disease, a precise estimate of its global incidence is meaningless. ASC-US usage is generally a reflection of a number of factors relating to cervical screening. It can be surmised that the highest ASC-US rates will be seen in screening programs that attach greater importance to screening sensitivity as opposed to specificity, in programs with extended screening intervals, in countries with immature screening programs and possibly in countries where litigation for screening errors is prevalent.
- **Age**
  ASC-US (or its equivalent) is in use for all women who are eligible for cervical screening, the age range for which varies from one country to another. Generally, a wide age range may be affected, from teenagers to elderly women.
- **Sex**
  ASC-US terminology is relevant only in females.
- **Site**
  ASC-US terminology is used exclusively in the reporting of cervical and vaginal samples.
- **Treatment**
  Treatment for ASC-US is generally not recommended unless the cytological changes are persistent or until a definitive neoplastic lesion has been diagnosed histologically or colposcopically. Even when neoplasia is subsequently identified, it may be prudent to adopt a watch-and-wait policy (cytological surveillance) as regression of cervical neoplasia is common. The decision whether to treat a woman with a histologically confirmed lesion following an ASC-US report rests with the clinician and the woman herself, taking into account factors such as the severity of the confirmed disease, age, reproductive status, the extent and type of treatment proposed, and whether there are any contraindications to such treatment.
- **Outcome**
  There is considerable variation in the reported outcome of ASC-US reports, which is no doubt a reflection of the subjective nature of cytology and the poor interobserver variability associated with equivocal cytology reports. The Bethesda System has undergone two modifications since its inception in 1988, making it difficult to compare the results of different studies. In one study of 4,143 cases of ASCUS, SIL of any grade was subsequently demonstrated in 63% of women (Jones and Novis 1996). In contrast, a separate investigation of 560 women with ASCUS found SIL in 36% of cases (Lachman and Cavallo-Calvanese 1998). Despite such variations,

there can be no doubt that ASC-US is an important contributor to high-grade cervical disease.

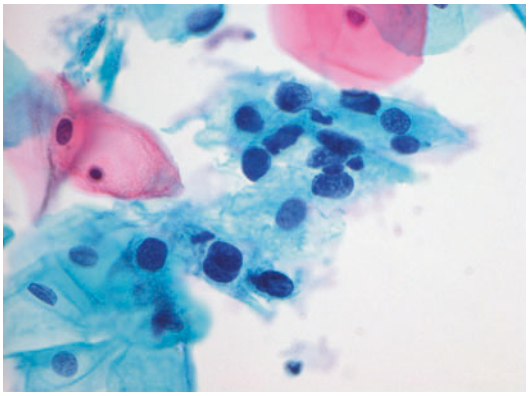
Macroscopy

There are no reliable macroscopic (colposcopic) features of ASC-US.

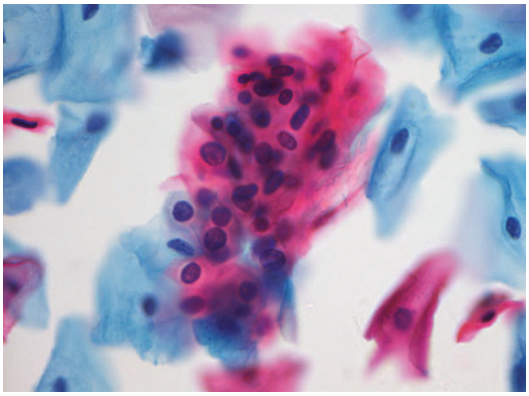
Microscopy

Although many attempts have been made to describe the cytomorphological features of ASC-US, all are plagued by interobserver variations, subjectivity of interpretation, and lack of consistency. The problem of defining reliable criteria is compounded by cellular artifacts caused by differences in specimen preparation techniques and methods of cell fixation. ▶ Liquid-based cytology has, to a certain extent, helped to improve the consistency of cell preparations but has not had any appreciable effect on ASC-US reporting rates.

With the above caveats, ASC-US cells can be described in terms of the type of cells affected and the extent to which cell nuclei deviate from normality. In the majority of cases, the affected cells are mature squamous epithelial cells which show a two- to three-fold increase in nuclear size, mild hyperchromasia, slight coarsening of the nuclear chromatin, and minor irregularities in the nuclear membrane (Figs. 1–4). The changes, however, fall short of low-grade or high-grade SIL.



Atypical Squamous Cells of Undetermined Significance (ASC-US), Fig. 1 ASC-US cells showing mild nuclear hyperchromasia that may be related to cell degeneration



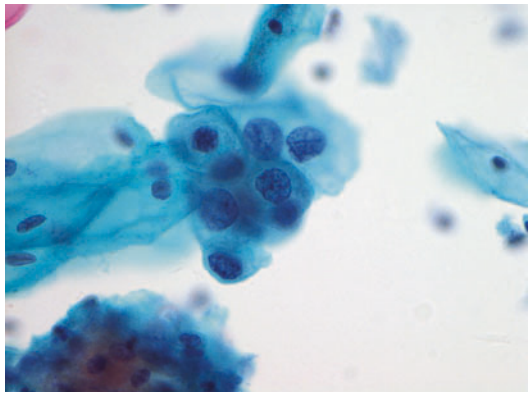
Atypical Squamous Cells of Undetermined Significance (ASC-US), Fig. 2 ASC-US cells showing nuclear hyperchromasia and slight coarsening of nuclear chromatin. These changes are often seen in chronic inflammatory conditions

Immunophenotype

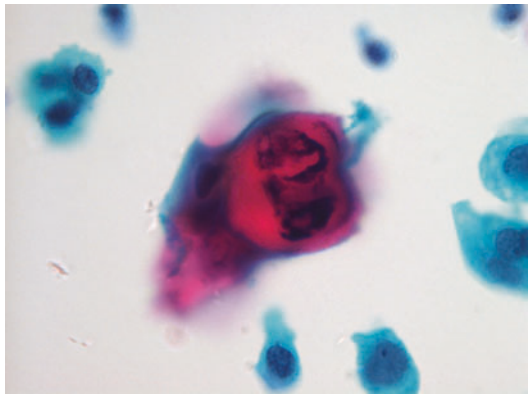
The broad and equivocal nature of ASC-US renders futile any attempt to define the immunophenotype of this cytological entity. Nevertheless, immunocytochemical studies continue to search for reliable biomarkers that may aid in the triage of ASC-US cases. Important candidate markers include high-risk human papillomavirus (HPV), p16(INK4a), Ki-67, MCM, and CDC proteins.

Molecular Features

The molecular features of ASC-US are diverse, reflecting the underlying pathological and non-pathological entities that this category represents. ASC-US cases with confirmed outcomes of SIL or cancer can be expected to harbor molecular alterations associated with HPV infection, including changes in the expression of p16(INK4a), cyclin E, MCM, TOP2A, and other genes that regulate the cell cycle.



Atypical Squamous Cells of Undetermined Significance (ASC-US), Fig. 3 The nuclear irregularities seen here are considered sufficient to warrant a report of low-grade SIL rather than ASC-US



Atypical Squamous Cells of Undetermined Significance (ASC-US), Fig. 4 Degenerative cell changes associated with invasive disease can lead to an underestimation of the severity of the underlying lesion

Differential Diagnosis

ASC-US is an undesirable output of cervical screening and its usage should be kept to a minimum. In practice, there should be very few cases in which it is not possible to classify a cervical sample as clearly normal or neoplastic. Cases that defy accurate classification require careful consideration of differential diagnoses before an ASC-US report is issued. The most common differential diagnosis is low-grade SIL (Fig. 3). More rarely, high-grade SIL or even invasive cancer can present as equivocal morphological changes (Fig. 4).

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Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H)

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Synonyms

Borderline changes, high-grade dyskaryosis not excluded

Definition

The term “atypical squamous cells” (ASCs) is used by cytologists to describe equivocal morphological changes in squamous epithelial cells of the cervix. The term is specific to the Bethesda terminology, which is a widely recognized classification system for reporting cervical cytology (Solomon et al. 2002). The Bethesda System (TBS) stresses the importance of qualifying an ASC diagnosis into “atypical squamous cells of undetermined significance” (ASC-US) and

"atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion" (ASC-H).

Morphological changes in cervical epithelial cells lie on a spectrum from the perfectly normal to definitely abnormal (neoplastic). The nature of cervical cytology is such that there will always be a proportion of cases for which doubt exists over the significance of morphological changes seen in one or more cells. The ASC-H category conveys the message to the clinician that there is genuine uncertainty as to whether the changes result from benign processes or from a high-grade squamous intraepithelial lesion (SIL). Colposcopy should always be advised in these circumstances. Studies reporting on clinicopathological outcomes have shown that the proportion of ASC-H associated with high-grade lesions exceeds that of the ASC-US category (Sherman et al. 1999, 2001).

Clinical Features

- **Incidence**
  ASC-H is less common than ASC-US, accounting for 5–10% of ASC cases. However, the underlying risk of a high-grade lesion is higher for ASC-H than for ASC-US.
- **Age**
  ASC-H is in use for all women who are eligible for cervical screening, the age range for which varies from one country to another. Generally, a wide age range may be affected, from teenagers to elderly women.
- **Sex**
  ASC-H terminology is relevant only in females.
- **Site**
  ASC-H terminology is used exclusively in the reporting of cervical and vaginal samples.
- **Treatment**
  Treatment for ASC-H depends upon the severity of the underlying lesion, which varies considerably. The decision whether to treat a woman with a histologically confirmed lesion following an ASC-H report rests with the clinician and the woman herself, taking into account factors such as the severity of the confirmed disease, age, reproductive status, the extent and type of treatment proposed, and whether there are any contraindications to such treatment.
- **Outcome**
  There is considerable variation in the reported outcome of ASC-H reports, which is no doubt a reflection of the subjective nature of cytology and the poor interobserver variability associated with equivocal cytology reports. The Bethesda System has undergone two modifications since its inception in 1988, making it difficult to compare the results of different studies. However, the underlying risk of a high-grade lesion is higher for ASC-H than for ASC-US. The positive predictive value of ASC-H for a high-grade lesion lies in the range 24–40% (Sherman et al. 1999, 2001). There is therefore no doubt that ASC-H is an important contributor to high-grade cervical disease.

Macroscopy

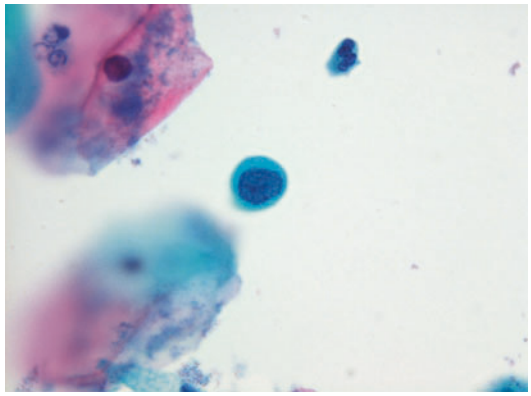
There are no reliable macroscopic (colposcopic) features of ASC-H.

Microscopy

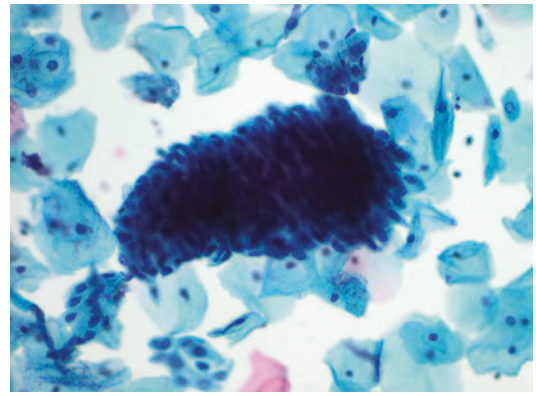
ASC-H cells share many of the cytological features of high-grade SIL but lack sufficient criteria for definitive classification. The affected cells are generally small with an increased nucleocytoplasmic ratio, and may present as single cells (Figs. 1 and 2) but also commonly as hyperchromatic crowded cell groups (Fig. 3).

Immunophenotype

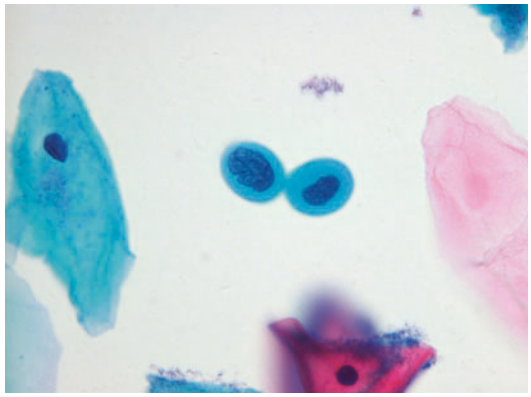
The broad and equivocal nature of ASC-H renders futile any attempt to define the immunophenotype of this cytological entity. Nevertheless, immunocytochemical studies continue to search for reliable biomarkers that may aid in the more definitive classification of entities currently reported as ASC-H. Important candidate markers include high-risk human papillomavirus (HPV),



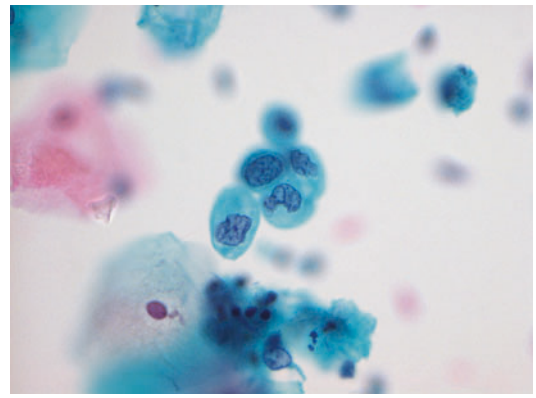
Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H), Fig. 1 Single ASC-H cell. Note high nucleocytoplasmic ratio but bland nuclear chromatin



Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H), Fig. 3 Hyperchromatic crowded group of ASC-H cells. It is impossible to discern the nuclear chromatin pattern in this tightly packed group of cells



Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H), Fig. 2 Single ASC-H cells. Note undulating nuclear borders but bland nuclear chromatin



Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H), Fig. 4 High-grade SIL. The nuclear borders and chromatin in these small cells are clearly irregular

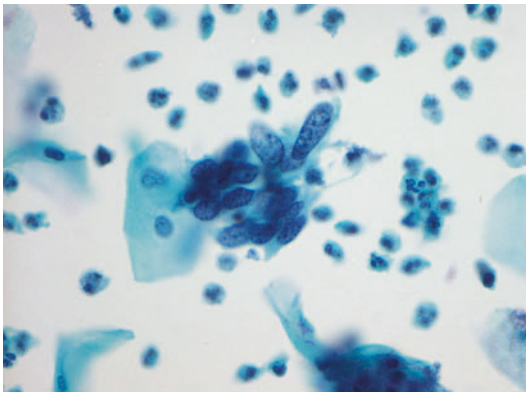
p16(INK4a), Ki-67, MCM (Mini Chromosome Maintenance), and CDC (Cell Division Cycle) proteins.

Molecular Features

The molecular features of ASC-H are diverse, reflecting the underlying pathological and non-pathological entities that this category represents. ASC-H cases with confirmed outcomes of SIL or cancer can be expected to harbor molecular alterations associated with HPV infection, including changes in the expression of p16(INK4a), cyclin E, MCM, TOP2A, and other genes that regulate the cell cycle.

Differential Diagnosis

ASC-H is an undesirable output of cervical screening and its usage should be kept to a minimum. In practice, there should be very few cases in which it is not possible to classify



Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H), Fig. 5 Invasive squamous cell carcinoma in a Pap smear. Note nuclear pleomorphism and coarsely granular chromatin

a cervical sample as clearly normal or neoplastic. Cases that defy accurate classification require careful consideration of differential diagnoses before an ASC-H report is issued. The most common differentials include high-grade SIL (Fig. 4) and invasive cancer (Fig. 5).

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B

Barrett's Esophagus, Cytological Findings

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Synonyms

Columnar-lined esophagus, Barrett's metaplasia; Esophageal columnar metaplasia

Definition

In 2008, the Practice Parameters Committee of the American College of Gastroenterology defined Barrett's esophagus as a change in the distal esophageal epithelium of any length that can be recognized as columnar type mucosa at endoscopy and is confirmed to have intestinal metaplasia by biopsy of the tubular esophagus (Wang and Sampliner 2008). While in Japan, Barrett's esophagus is defined simply as metaplastic columnar-lined esophagus that is macroscopically recognizable (Takubo et al. 2009).

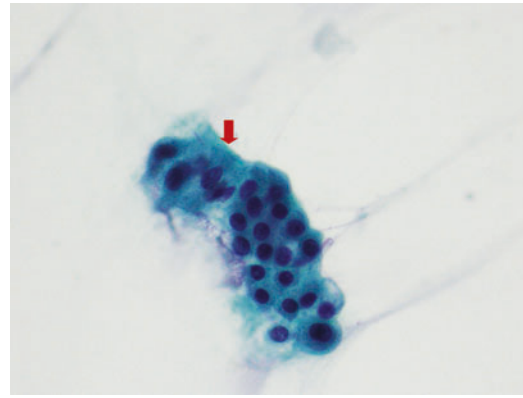
Clinical Features

- **Incidence**
  Barrett's esophagus continues to be increasingly recognized and is believed to be the major risk factor for the development of esophageal adenocarcinoma. It is believed that 10–14% of patients with long-standing gastroesophageal reflux disease will have Barrett's esophagus and it is the sole known precursor of esophageal adenocarcinoma.

  There are no current risk factors recognized to identify asymptomatic patients although some studies reveal that increased body mass index is correlated with Barrett's esophagus.
- **Age**
  Incidence is higher in patients with long-standing heartburn aged 40–50 years.
- **Sex**
  The typical patient is a Caucasian male with long-standing gastroesophageal reflux disease. Males have an incidence 10x the females
- **Site**
  Intestinal metaplasia occurs in the lower esophagus at the esophagus-gastric junction, which has not been easy to define. During endoscopic examination, the squamous-columnar junction moves slightly with inspiration and expiration. For that reason, most Japanese endoscopists believe that the lower end of the palisade vessels is a more suitable definition of the esophagus-gastric junction. Gastroenterologists in North America and Europe support the definition of the esophagus-gastric junction as being at the proximal margin of the gastric mucosal folds (Takubo et al. 2009).

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4

- **Treatment**
  There is no need for local treatment unless high-grade dysplasia is found. Several endoscopic resections are in use but other mucosal ablation techniques have been advocated.
- **Outcome**
  The goal of surveillance in patients with Barrett's esophagus is the detection of dysplasia and early cancer. Dysplasia occurs on the background of metaplasia – a fundamental and distinctive change in the epithelium of the esophagus from one differentiated cell type to another. Dysplasia is the best current indicator for the risk of cancer. Cytology can play a role in this setting, to screen the patients that are at risk.



Barrett's Esophagus, Cytological Findings, Fig. 1 Sheet partially folded of glandular cells with a villous border. (Pap stain)

Macroscopy

Barrett's mucosa appears like a red velvety area that protrudes in a diffuse or focal manner above the esophagus-gastric junction. It merges imperceptibly with the gastric mucosa. The transition of the metaplastic mucosa to the squamous epithelium may be a straight or irregular line, like the transition of the non-metaplastic epithelium, or can be found islands of pink squamous epithelium within the red mucosa.

The diagnosis should always be confirmed histologically.

Microscopy

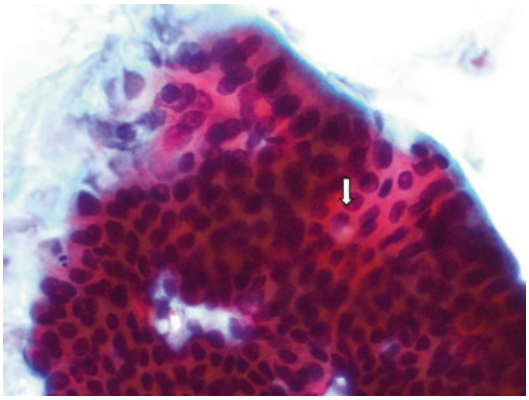
Endoscopy with multiple systematic biopsies is needed to establish the diagnosis of Barrett's esophagus. The reason to do multiple biopsies in the columnar appearing esophagus is to identify the presence of intestinal metaplasia. Three types of epithelium are recognized in Barrett's metaplasia: oxyntico-cardiac, cardiac, and intestinal. Oxyntico-cardiac and cardiac types are identical to the corresponding gastric epithelial regions and one should be cautious in rendering a diagnosis of Barrett's metaplasia in lower esophagus samples (Schmitt and Oliveira 2010).

To demonstrate intestinal type mucosa by biopsy, multiple specimens (more than 8) are often needed. If intestinal metaplasia is focal, then it may not always be seen in biopsy specimens.

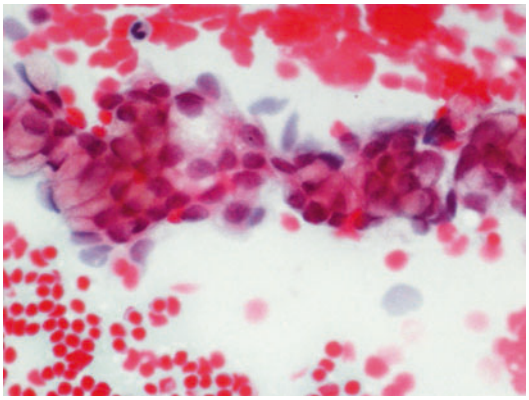
Endoscopic brush cytology has been used during surveillance of Barrett's esophagus as it increases the ability to sample the cells and leads to better diagnoses (Takubo et al. 2009); (Schmitt and Oliveira 2010).

Cytologically, intestinal type metaplasia is identified when goblet cells are present along with columnar or cuboidal cells. A considerable amount of epithelium folded or in sheets is necessary for recognition of the "portholes" which are mucus cells seen from above/below (Schmitt and Oliveira 2010). The villous border is more difficult to demonstrate in smears (Fig. 1).

Studies are conflicting as to how much additional information can be obtained from cytological examination. However, the use of new genetic markers, such as fluorescent in situ hybridization, may be promising in increasing the clinical utility of brush cytology (Lao-Sirieix et al. 2007). Criteria favoring dysplasia are enlarged nucleus, delicate chromatin, and apparent nucleoli (Fig. 2). With increasing dysplasia, groups of cells may have loss of polarity (Fig. 3).



Barrett's Esophagus, Cytological Findings, Fig. 2 Sheet of glandular cells with slightly enlarged nucleus. Note the "porthole" of a goblet cell (*arrow*). (Pap stain)



Barrett's Esophagus, Cytological Findings, Fig. 3 Group of epithelial mucinous cells with loss of polarity. (Pap stain)

Immunophenotype

Gastric and intestinal mucosa are protected by a mucus layer of high molecular weight glycoproteins synthesized by normal epithelial cells in a cell- and tissue-specific pattern (Chaves et al. 2007). MUC5AC and MUC6 are the protein cores of gastric mucus, foveolar and mucopeptic, respectively. MUC2 and CD10 stain the columnar intestinal cells. Goblet cells are MUC1 and MUC6 positive.

Molecular Features

Barrett's esophagus is phenotypically and genotypically heterogeneous formed by several distinct genetic clones, and chromosomal instability is present in Barrett's esophagus even in the absence of dysplasia or cancer. Columnar nongoblet and goblet cells have chromosomal gains and the two metaplastic phenotypes, gastric and intestinal, are equally involved (Chaves et al. 2007).

Over expression of EGFR might be related to demonstrated abnormalities in chromosome 7.

It is known that expression of cyclin A at the luminal surface is increased throughout the metaplasia-dysplasia-carcinoma sequence. This fact has been studied to differentiate dysplastic from non-dysplastic epithelium in cytological material (Lao-Sirieix et al. 2007).

Differential Diagnosis

Esophagitis can mask Barrett's esophagus and therapy should be given prior to endoscopy.

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Basal Cell Adenoma of Salivary Gland, Cytological Findings

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Synonyms

Tubular adenoma, trabecular adenoma, dermal analogue tumor, basaloid adenoma, and monomorphic adenoma.

Definition

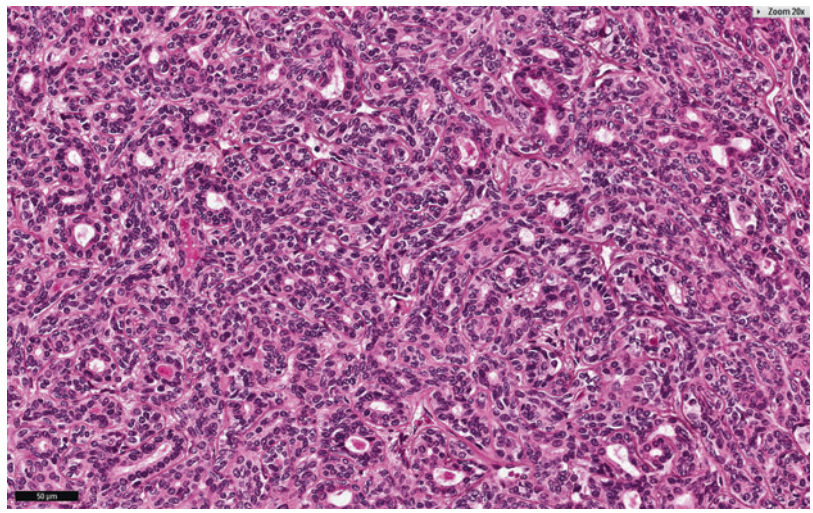
Basal cell adenoma (BCA) (Barnes et al. 2005; Ellis and Auclair 2008) is a salivary adenoma composed of monotonous dark epithelial cells with solid, trabecular, tubular, and membranous types (Fig. 1).

Clinical Features

- **Incidence**
  BCA represents approximately 3% of salivary gland tumors and is the last of three most common salivary adenomas after pleomorphic adenoma and Warthin's tumor.
- **Age**
  BCA may be seen in children, but its peak of incidence is between 70 and 80 years.
- **Sex**
  There is 1.5 to 1 for female to male predominance.
- **Site**
  Most of BCAs occur in the parotid gland. Upper lip is the most common site for canalicular adenoma.
- **Treatment**
  Surgical treatment is a treatment of choice.
- **Outcome**
  Recurrences are exceptional.

Macroscopy

BCA is medium-sized (1–2 cm), well-circumscribed tumors with firm and homogenous, gray to white cut-surface.



Basal Cell Adenoma of Salivary Gland, Cytological Findings, Fig. 1 Basal cell adenoma of the parotid gland. Dense proliferation of basaloid, dark cells (H&E stain)

Microscopy

Smears in basal cell adenomas are usually hypercellular and cell-rich and stroma-poor (Klijanienko et al. 1999). BCA belongs to the group of tumors exhibiting predominant epithelial/basal cell morphology. Smears show epithelial cells, numerous naked nuclei, and nonspecific connective fragments. Cells are small ovoid, basal and dark, isolated, or in cohesive clusters. Cytoplasm is narrow and basophilic. Nuclei contain bland chromatin without nucleoli. Usually, three-dimensional clusters show peripheral palisading. In some cases, whirling cells with squamous differentiation or pseudopapillary structures adjacent to hyaline globules are seen. Stroma is scant and fibrous. Naked nuclei, keratin debris, and occasionally squamous cells may be observed (Fig. 2). Chondromyxoid stroma or plasmacytoid myoepithelial cells are absent.

Immunophenotype

Luminal cells of BCA are immunoreactive for cytokeratins, CEA, and EMA. Myoepithelial cells may be evidenced using SMA. Most cells are reactive for S-100.

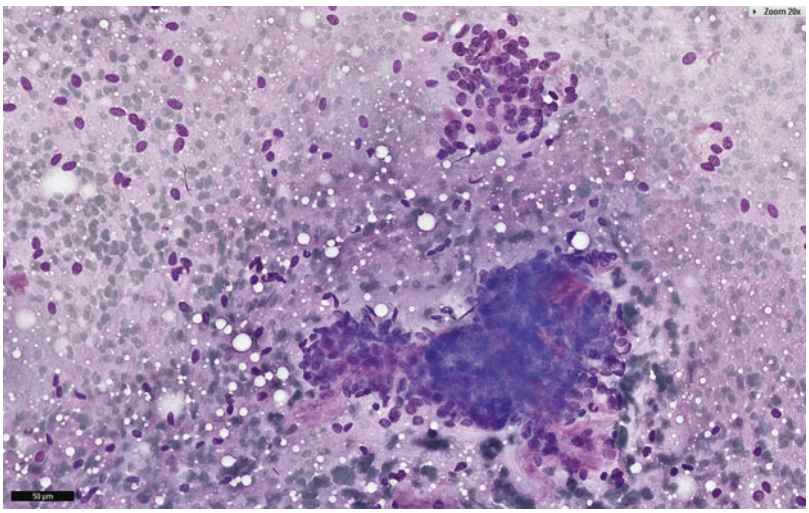
Molecular and Cytogenetic Features

Loss of heterozygosity (LOH) at 16q12-13 has been described in some membranous variants of BCA.

Differential Diagnosis

BCA should be differentiated from other basal cell tumors such as canalicular adenoma, basal cell adenocarcinoma (BCAc), and poorly differentiated (solid-type) adenoid cystic carcinoma (ACC) and from other tumors with predominant myoepithelial cell differentiation: pleomorphic adenoma (PA), polymorphous low-grade adenocarcinoma (PLGA), and epi-myoepithelial carcinoma (EMEC).

Canalicular adenoma is cytologically identical with BCA. Usually, canalicular adenoma is localized in the upper lip. BCAc constitutes a major differential diagnostic problem due to its cytomorphological similarity with BCAd. Tumor showing basaloid features, cytonuclear pleomorphism, large three-dimensional cellular clusters, mitotic figures, and necrosis may be adenocarcinoma (Klijanienko et al. 1999). ACC, cellular PA, PLGA, and EMEC may contain hyaline globules intimately associated with basaloid features. Search for tubular and papillary structures (ACC, PLGA, and EMEC), clarified nuclei (PLGA), clear cells



Basal Cell Adenoma of Salivary Gland, Cytological Findings, Fig. 2 Basal cell adenoma. Small roundish cell with basaloid morphology. Nonspecific connective fragments and naked nuclei. Chondromyxoid and myoepithelial cells are absent (MGG stain)

(EMEC), and plasmacytoid myoepithelial cells with well-delineated cytoplasm (PA) is mandatory (Klijanienko and Vielh 1996, 1997, 1998a, b). Moreover, PAs always show chondromyxoid stroma which is absent in BCAds.

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Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings

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Synonyms

BCC

Definition

Non-melanocytic skin tumor that arises from epidermal basal cell.

Clinical Features

- **Incidence**
  The most frequent malignant skin tumor.
- **Age**
  Middle-old age
- **Sex**
  No gender predilection
- **Site**
  Head and neck; eyelid
- **Treatment**
  Surgery, chemotherapy, and radiotherapy
- **Outcome**
  Favorable. BCCs rarely metastasize, but grow slowly, eroding surrounding tissues, proximal structures, and even the globe.

Macroscopy

BCC arises as a single lesion after prolonged exposure to sunlight or as multiple lesions in patients with xeroderma pigmentosum. The clinical presentation of BCC may be nodular and/or ulcerative or sclerosing (morphea type).

Microscopy

Adequate scraping should reach the dermis. Smears generally show dense compact cell groups in which, often, cytological details can be observed only at the edges of fragments. Here, the nuclei may be organized in regular lines, perpendicular to the group, producing a "picket fence pattern." Cells are poorly differentiated with scanty or absent cytoplasm and oval nuclei with dense, compact, chromatin without nucleoli; mitoses are scanty, if present at all. In some cases, macrophages engulfed with hemosiderin or melanin are distributed across the background, representing the cytological aspect of "pigmented" BCC. In others, some nuclei are larger, with coarse chromatin and evident nucleoli; the cytoplasm is larger and denser than usual and stains orange with the Papanicolaou stain. In these cases, the

differential diagnosis includes Bowen disease and ▶ squamous-cell carcinoma.

Differential Diagnosis

Bowen disease and squamous-cell carcinoma

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Benign Orbital Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Description

▶ Schwannoma and hemangioma are other relatively common benign orbital tumors. Imaging diagnosis of both is generally quite accurate, and hence, cytological diagnosis is seldom requested. The cytological features of schwannoma are almost identical to those described at other sites showing a variable amount of spindle cells; either isolated or organized in small dense groups, these latter may occasionally show a "picket fence" arrangement at the edge of the groups and single cells often show bent, "comma-shaped" nuclei. Smear FNC is not usually requested for vascular lesions because it is poorly informative and carries a risk of hemorrhage; FNC of orbital leiomyoma has been also described.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects

- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings

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Description

Bacteria, fungi, and other organisms may cause inflammatory lesions which rarely need cytological diagnosis. Aspiration may produce quite nonspecific granulocyte-rich smears and necrotic tissue fragments. Orbital aspergillosis, cryptococcosis, and cysticercosis have been described cytologically. Granulomatous lesions (▶ tuberculosis or sarcoidosis) may also occur; their cytological patterns are indistinguishable and do not differ from that seen at other sites. The rupture of dermoid cysts or other foreign bodies may cause reactive granulomatous infiltrates. Wegner's granulomatous vasculitis lesions may occur, being cytological features indistinguishable from those of other pathologies. Orbital involvement by ▶ Langerhans cell histiocytosis or sinus histiocytosis with massive lymphadenopathy (SHML) has also been reported; the presence of histiocytes exhibiting emperipolesis is the key element suggesting a diagnosis of SHMH.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings

- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
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- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Biliary Duct Brushing

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Synonyms

Brush cytology of extrahepatic bile ducts

Definition

Biliary duct brushing is an adjuvant procedure to endoscopic retrograde cholangiopancreatography (ERCP), a technique that combines endoscopy and fluoroscopy. It collects cells for cytological diagnosis and ancillary testing. This is the most widely used technique in the diagnosis of biliary strictures.

Principle

Biliary duct lesions generally are not accessible to biopsy.

Duodenal contents were used since 1920 for cytological analysis but with low sensitivity. Collecting bile and pancreatic juice was possible through endoscopic retrograde cholangiopancreatography. The samples had low cellularity and showed artifacts from degenerative changes, rendering a low sensitivity. They may be used with brush cytology, improving overall sensitivity. Cytology samples from extracted stents and stent retrieval devices have low sensitivity for the same reasons (Volmar et al. 2006).

Brush cytology allows direct sampling of the lesion and strictures, which can be hindered by tumor desmoplasia, submucosal location of tumors or compression of extrinsic tumors.

Methodology

Through the endoscope, a guide wire is introduced in the biliary duct and a brush passed over the wire and drawn back and forth along the stricture. Multiple samples improve sensitivity and allow separation of material for glass slides, liquid-based preparation methods, and ancillary tests. Smears can either be air-dried for MGG stain or alcohol fixed for Pap stain. The brush tip can be centrifuged within the liquid fixative, improving cell yield. Immediate fixation prevents degenerative changes in cells, improving general sensitivity.

Quality Aspects

The combination of smears and liquid-based cytology improves general accuracy that has been reported to be 75.7% with 52.6% sensitivity, 99.4% specificity, 98.9% positive predictive value, 67.1% negative predictive value (Volmar et al. 2006). Factors that influence the results are interpathologist variation of interpretation and clinical information. Most false-negative results are sampling errors followed by interpretative and technical errors (Logrono et al. 2000).

Dilatation of stricture, increased stiffness, or elongation of brush does not influence sensitivity.

Adjuvant molecular and genetic analyses increase sensitivity but lower specificity.

Applications

This technique is most useful to study extrahepatic biliary and pancreatic obstructions. The more frequent symptom related is jaundice. Abnormalities of the extrahepatic biliary tract can be caused by inflammatory processes, malignancy, and calculus, diseases that should be accurately diagnosed prior to therapy. Most benign strictures are managed conservatively with ductal dilatation and stenting while malignant strictures may be treated by Whipple procedure, bile duct resection, or simple stenting if the patient's disease is unresectable. Recent advances in neoadjuvant chemo- and radiotherapeutic approaches underscore the importance of accurate preoperative diagnosis.

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Bladder Wash Specimen

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Synonyms

Bladder barbotage; Bladder wash cytology; Bladder irrigation cytology

Definition

Bladder washing has become the most important method to obtain urothelial cells at the time of diagnostic cystoscopy in patients with suspicion of bladder cancer and during follow-up cystoscopies in patients under follow-up surveillance. In academic centers, the latter make the majority of all urinary specimens. Bladder washings are much more cellular than voided urines, and the cells are much better preserved, allowing for more accurate diagnosis. The high recurrence rate, the intensive surveillance strategies, and the expensive treatment costs make bladder cancer the cancer with the highest cost per patient.

Principle

Bladder washing by repeated instillation of the same volume of normal saline or Ringer's solution is used to obtain cellular specimens of well-preserved urothelial cells from the surface of the bladder mucosa.

Methodology

After cystoscopy, the bladder is first completely emptied over a carefully inserted catheter after flexible cystoscopy or less commonly over a rigid cystoscope in order to discard detritus and degenerated cells. Using a syringe filled with about 60–100 ml, the bladder is washed over the catheter or the rigid cystoscope by forced instillation and gentle aspiration of the same volume of saline or Ringer's solution for about ten times. Care has to be taken not to aspirate the bladder wall. Fresh washing fluid should be transported immediately to the laboratory and processed to cytological specimens within 1–3 h. In case it cannot be delivered to the cytology laboratory within 3 h after sampling, one may consider prefixation of the cells by adding an equal volume of 50% ethanol solution. This preserves the cells for up to 3 days and prevents bacterial overgrowth. Adding formalin is not recommended as it fixes rather than conserves the cells and prevents them from attaching to the surface of the glass slide. Preparation of cytospins from the sediments of the bladder washings is the preferred method because cytospins provide crisp morphological details on a small area. Two cytospin specimens are considered as representative. The preparation of regular smears is reasonable in case of abundant sediment, but not recommended otherwise. Fluid-based cytology preparations (e.g., ThinPrep®, UroCyte®, or SurePath®) are a valid alternative, although they lead to increased costs without clearly improving the quality as compared to cytospins. Cytological specimens of the urinary tract should be stained according to Papanicolaou or by hematoxylin-eosin in order to highlight the nuclear details that are critical for cytological diagnosis.

Quality Aspects

Ideally, all available fluid should be sent to the laboratory. The details of the nuclear features are critical for an accurate diagnosis. Therefore, immediate fixation of the cytospin specimens is important in order to avoid drying of the cells. Numerous bacteria without presence of granulocytes indicate secondary bacterial overgrowth due to suboptimal conditions during the transport, such as long delay, high temperature, and no adding of 50% ethanol for preservation. Admixed fresh blood that may obscure the morphology of urothelial cells is not a common problem.

Applications

The main purpose of bladder washing is the detection of urothelial carcinoma in situ (CIS) or invisible carcinomas (e.g., in diverticula) during surveillance of patients with known urothelial carcinoma. In patients with newly detected urothelial carcinoma, bladder washing prior to tumor resection can detect simultaneous high-grade lesions (e.g., CIS) that might be overlooked otherwise.

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Bone Cyst (Aneurysmal Bone Cyst and Solitary Bone Cyst), Cytological Findings

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Synonyms

Aneurysmal bone cyst (ABC); Primary aneurysmal bone cyst; Simple bone cyst; Solitary bone cyst (SBC); Unicameral bone cyst

Definition

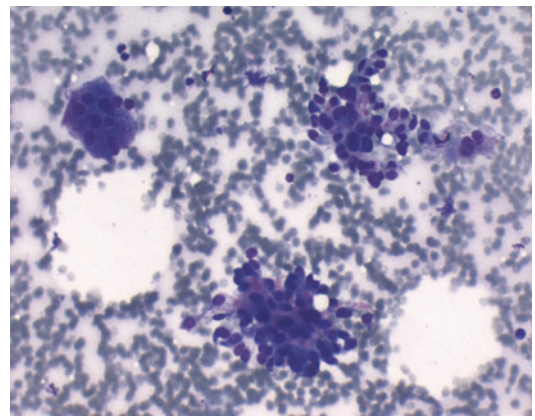
1. ABC: benign, multilocular cyst with rich capillary tissue with a locally destructive growth.
2. SBC: benign unilocular cyst with serous fluid and no tumor tissue.

Clinical Features

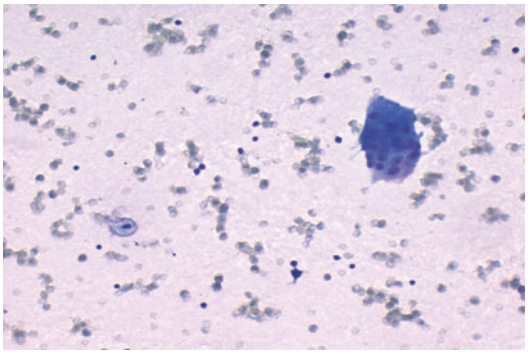
- **Incidence**
  1. ABC: 2.5% of all primary bone tumors.
  2. SBC: relatively common.
- **Age**
  1. ABC: most patients are younger than 20 years.
  2. SBC: a majority of patients are younger than 15 years.
- **Sex**
  1. ABC: no sex predilection.
  2. SBC: males dominate (2:1).
- **Site**
  1. ABC: all bones can be affected, but the bones around the knee and the vertebral column are overrepresented.
  2. SBC: the humerus and femur dominate but most bones can present the lesion.
- **Treatment**
  1. ABC: curettage and bone grafting.
  2. SBC: curettage and bone grafting, instillations.
- **Outcome**
  1. ABC: most patients are cured.
  2. SBC: most patients are cured.

Microscopy

1. ABC: the FNA biopsy yields a yellow clear fluid with varying amount of peripheral blood. The smears show fragments of monomorphic spindle cells, giant cells of osteoclast type, and macrophages (Fig. 1). Osteoid and osteoblasts are sometimes seen.
2. SBC: the FNA biopsy material is dominated by a clear yellowish fluid, macrophages, inflammatory cells, and osteoclasts which are often present in the smears (Fig. 2).



Bone Cyst (Aneurysmal Bone Cyst and Solitary Bone Cyst), Cytological Findings, Fig. 1 Aneurysmal bone cyst (ABC): FNA smear show one osteoclast and two fragments of monotonous round to oval cells with a delicate pinkish stroma. Rich admixture of red blood cells. MGG



Bone Cyst (Aneurysmal Bone Cyst and Solitary Bone Cyst), Cytological Findings, Fig. 2 Solitary bone cyst: smear from aspirated clear fluid show a giant cell of osteoclastic type and a macrophage in a background of a thin protein rich fluid. Few red blood cells present. MGG

Differential Diagnosis

1. ABC: telangiectatic osteosarcoma, giant cell tumor, giant cell reparative granuloma.
2. SBC: cystically degenerated solid tumors such as giant cell tumor and fibrous dysplasia.

References and Further Reading

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Breast Cysts, Cytological Findings

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Synonyms

Fibrocystic changes

Definition

Breast cysts appear in the context of fibrocystic changes (FCC). FCC encompass a group of benign breast changes that are considered to represent normal, but exaggerated, hormonally mediated breast tissue responses. They are the most common benign breast condition and frequently the causes of a palpable breast lump, frequently a cyst.

Clinical Features

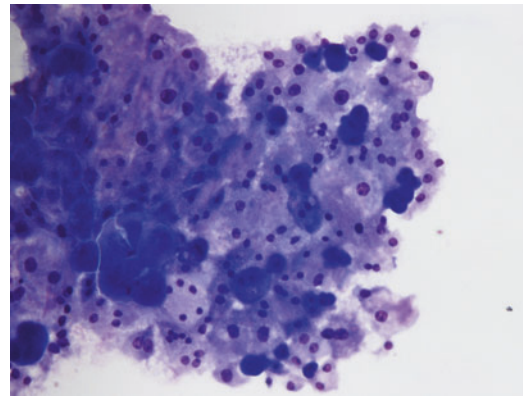
- **Incidence**
  More than one-third of women between the third and fifth decades of life show some evidence of FCC.
- **Age**
  20–45 years of age.
- **Sex**
  More frequent in women.
- **Site**
  Although FCC are frequently multifocal and bilateral, often the initial presentation is as a solitary lesion.
- **Treatment/Outcome**
  The clinical manifestations are more prominent during the reproductive cycle, and in the absence of hormonal replacement therapy, the symptoms of FCC generally cease in the first 2 years post menopause.

Macroscopy

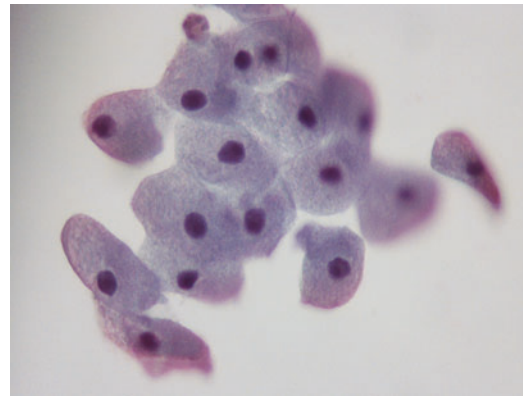
The cysts are better evaluated at ultrasound, show circumscribed margins, sharp anterior and posterior walls, no internal echoes and posterior enhancement. Cysts are called complex when contain internal echoes reflecting the presence of debris within the cyst fluid. Lesions that do not exhibit all the characteristics of a simple cyst require further workup, commonly with FNAC in clinical practice.

Microscopy/Cytology

The aspirated fluid from a breast cyst may be clear or turbid with variable color (yellowish, greenish, brownish). Such cysts have no epithelial lining and the cytological smears are characterized by an amorphous, proteinaceous material and variable amount of foam cells. Some cystic lesions of the breast may be lined by epithelium with apocrine metaplasia (apocrine cysts). These cysts generally exhibit a thick content and variable amount of apocrine cells isolated or arranged in groups of varying sizes. Apocrine cells usually are found arranged in cohesive monolayer sheets or as isolated, single cells (Figs. 1 and 2). Occasionally, nuclear atypia can be seen in apocrine cells. Ductal epithelium can be also present and is usually sparse and present as flat sheets.



Breast Cysts, Cytological Findings, Fig. 1 Apocrine cells in smears from FNA of a breast cyst (Giemsa stain)



Breast Cysts, Cytological Findings, Fig. 2 Apocrine cell details. Observe large and granular cytoplasm (Papanicolaou stain)

Differential Diagnosis

Other cystic lesions on the breast should be differentiated. Three-dimensional clusters may raise the possibility of the presence of an intracystic papillary lesion and need further investigation (see ▶ Papillary Tumors of the Breast, Cytological Findings). Mucocele-like lesions and mucinous carcinoma are another group of breast lesions that may present as cystic. Both present mucinous background, at contrast with breast cysts; mucocele-like lesions show scant cellularity, no or rare intact single epithelial cells, without atypia, and in monolayered arrangements. Mucinous carcinoma shows higher cellularity, more single tumor cells, three-dimensional cluster, and mild to severe nuclear atypia. Some metaplastic carcinomas can present as cystic lesions, but in these cases, besides the atypical tumor cells (that can be sparse), the background shows cellular debris. Epidermoid cysts may be found on the breast but the cytological aspect is similar of the other sites: creamy aspirate, anucleate squames, and keratinous debris.

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Bronchial Washing

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Definition

A bronchial washing is a procedure in which saline is inserted into a part of the lung and re-collected for further assessment.

Principle

A bronchoscope is passed through the mouth or nose into the lungs, and fluid (10–30 ml) is squirted into a small part of the lung and re-collected.

Methodology

The methodology of a bronchial washing is very similar to that of a bronchoalveolar lavage (BAL); however, the amount of fluid which is inserted into the airways is much less (10–30 ml).

Technical Procedure

A bronchoscope is introduced into the airways, mostly under local anesthetic. Ten to thirty milliliters of warmed isotonic saline is flushed through the operating channel of the bronchoscope. The fluid is aspirated again and collected into a receptacle.

Workup in the Laboratory

The volume is measured. Then mucus may be filtered by using a gaze tissue prior to centrifuging the fluid. Smears or cytospins are stained, usually with Papanicolaou. Additional slides may be prepared for further workup (e.g., immunocytochemistry, immunofluorescence, special stains). Infections can be diagnosed using immunofluorescence or special stains.

Applications

The aim of a bronchial washing is either the detection of malignant cells in patients with a suspicion of a malignancy or the gain of material for workup of infectious disease.

Table with Examples (Links)

Specific technique	Used for diseases like
Assessment of cytomorphology Immunocytochemistry	Malignant tumors
Immunofluorescence Special stains	Infection
Culture	Infection

References and Further Reading

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Bronchoalveolar Lavage Specimen

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Definition

Bronchoalveolar lavage (BAL) is a procedure in which saline is inserted into a part of the lung and re-collected for further assessment.

Principle

A bronchoscope is passed through the mouth or nose into the lungs, and fluid is squirted into a small part of the lung and re-collected.

Methodology

Technical Procedure

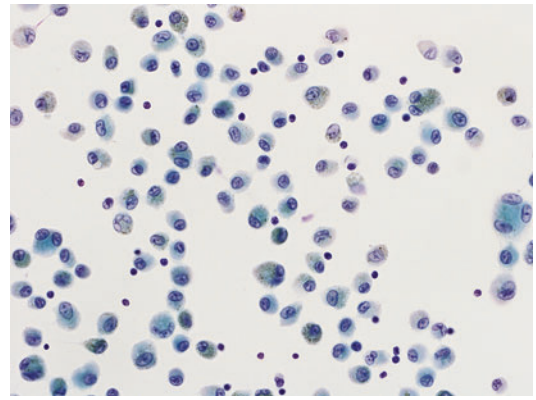
A bronchoscope is introduced into a segmental or subsegmental bronchus. There it is wedged to occlude the airways. Most often a flexible bronchoscope is used; however, the usage of an endobronchial tube or a rigid bronchoscope is possible. A BAL can be performed under local anesthetic or under general anesthesia. Hundred to three hundred milliliter of warmed isotonic saline is flushed through the operating channel of the bronchoscope in portions of 20–50 ml. The fluid is aspirated again and collected into a receptacle.

Workup in the Laboratory

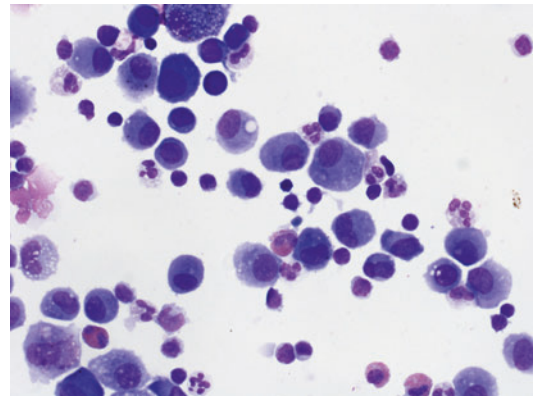
The volume is measured. Then mucus may be filtered by using a gaze tissue prior to centrifuging the fluid. The absolute number of cells is determined. Smears or cytospins are stained with Papanicolaou and MGG (see Figs. 1 and 2). The MGG-stained slide is used for the assessment of the differential cell count. Additionally, slides may be prepared for further workup (e.g., immunocytochemistry, iron stain, Sudan stain, etc.). The lymphocyte subpopulations (e.g., CD4 and CD8) can be analyzed by immunocytochemistry or flow analysis (see Figs. 3 and 4). Infections can be diagnosed using immunofluorescence or special stains.

Quality Aspects

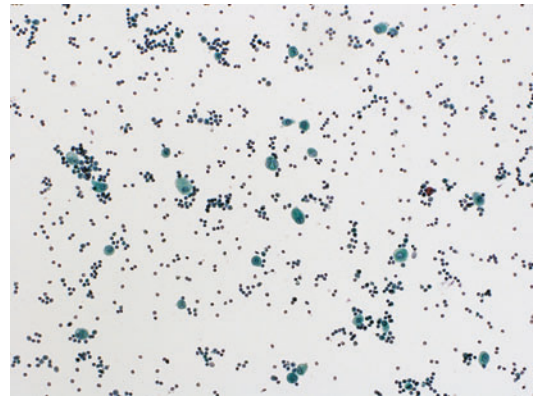
A re-collection of 40–70% of the applied fluid is desirable. The number of epithelial cells (e.g., squamous cells, bronchial cells) should not exceed 5% of all nucleated cells.



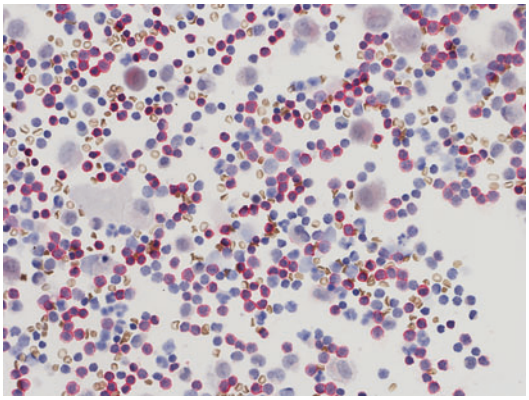
Bronchoalveolar Lavage Specimen, Fig. 1 A normal bronchoalveolar lavage shows mainly macrophages and occasional lymphocytes (BAL, Papanicolaou, 10×)



Bronchoalveolar Lavage Specimen, Fig. 2 The Giemsa stain facilitates the identification of mast cells and eosinophils (BAL, Giemsa stain, 40×)



Bronchoalveolar Lavage Specimen, Fig. 3 A marked lymphocytosis can be noted in patients with hypersensitivity pneumonitis (BAL, Papanicolaou, 4×)



Bronchoalveolar Lavage Specimen, Fig. 4 The CD4/CD8 ratio can be assessed using immunocytochemistry (BAL, ICC for CD4, 20×). In this example, the ratio was markedly decreased to 0.3

Applications

A BAL is mostly part of the workup of unknown interstitial lung disease. It may be used to diagnose infectious lung disease. It can be used to diagnose malignant tumors as well, depending on their location and distribution pattern.

Table with Examples (Links)

Specific technique	Used for diseases like:
Gross inspection	Alveolar proteinosis
Differential cell assessment	Interstitial lung disease
	Infection
Assessment of cytomorphology	Malignant tumors
Immunocytochemistry	
Immunofluorescence	Infection
Special stains	
Immunocytochemistry	Interstitial lung disease
Flow cytometry	
Culture	Infection

References and Further Reading

DeMay, M. (2007). *Practical principles of cytopathology* (Revised ed.). Chicago: American Society of Clinical Pathologists Press. ISBN-10: 0891895493, 13: 978-0891895497.

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Tötsch, M., Guzman, J., Theegarten, D., Schmid, K. W., & Costabel, U. (2007). Bronchoalveolar lavage. *Der Pathologe, 28*(5), 346–353.

C

Carcinoid Tumors of the Lung, Cytological Findings

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Definition

Carcinoid tumors include typical and atypical carcinoids and are characterized by their neuroendocrine morphology and expression of neuroendocrine markers. The distinction between typical and atypical carcinoids is based on histologic criteria: A typical carcinoid is a tumor with less than 2 mitoses per 2 mm^2, lacking tumor necrosis, and a size of at least 5 mm. An atypical carcinoid is a tumor with 2–10 mitoses per 2 mm^2 or with focal necrosis. Therefore, the distinction between typical and atypical carcinoids usually requires a surgical specimen.

Clinical Features

- **Incidence**
  Carcinoids are rare tumors and account for 1–2% of all lung cancers. 90% are typical carcinoids.
- **Age**
  There is a broad age range with a mean age at diagnosis of 45–55 years.
- **Sex**
  No sex predilection.
- **Site**
  Carcinoids can be localized throughout the lung. In 60% they are central, commonly with an endobronchial tumor component.
- **Treatment**
  Most carcinoids are treated by surgical resection. Treatment of metastatic disease is challenging as carcinoids are relatively resistant to chemotherapy and radiation. Somatostatin receptor-mediated radionuclide therapy of carcinoids with uptake on octreotide scan has shown some promising results.
- **Outcome**
  Typical carcinoids have an excellent prognosis with a 5-year survival rate of 92–100%. The prognosis of atypical carcinoids is worse with a 5-year survival rate of 61–88%. Additionally, the prognosis is stage dependent.

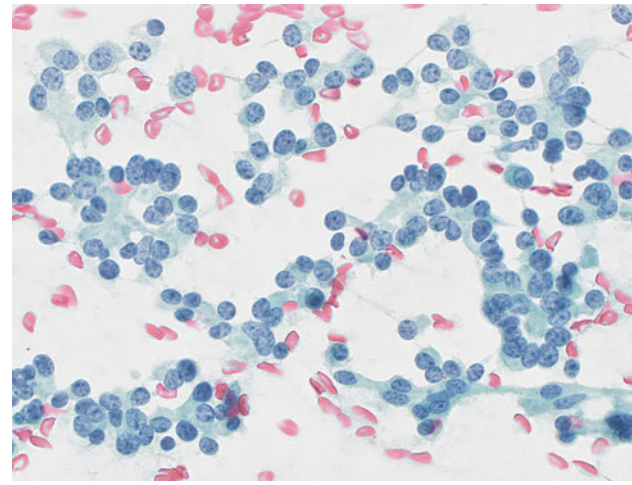
Macroscopy

On bronchoscopy central carcinoids typically present as endobronchial, highly vascular polypoid tumors, which tend to bleed.

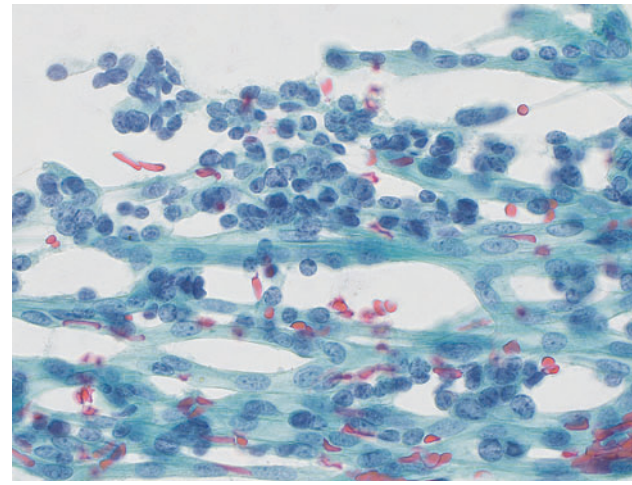
Microscopy

Typical carcinoids are characterized by a monotonous population of small uniform round to

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4



Carcinoid Tumors of the Lung, Cytological Findings, Fig. 1 Monotonous population of loosely cohesive cells with rosette-like aggregates. A few larger cells are present. Characteristic finely granular chromatin with inconspicuous nucleoli. The lobectomy specimen revealed a typical carcinoid (Fine needle aspiration of the lung, Papanicolaou stain, original magnification ×600)



Carcinoid Tumors of the Lung, Cytological Findings, Fig. 2 Same tumor like in Fig. 1 Carcinoid tumor with proliferation of streaming capillaries (Fine needle aspiration of the lung, Papanicolaou stain, original magnification ×600)

cuboidal, sometimes spindle-shaped cells with moderate amounts of finely granular cytoplasm and central or peripheral round to oval nuclei. A few larger, more atypical cells can be present (Fig. 1). Commonly the cells are individually dispersed or arranged in loose cohesive aggregates. In fine needle aspirates the cells are often arranged around prominent streaming proliferations of capillaries (Fig. 2).

The nuclei measure 7–10 μm, have a finely granular (salt-and-pepper) chromatin, an inconspicuous small nucleolus, and a smooth nuclear membrane. The cytoplasm is delicate and stripped nuclei may be present in a background of finely granular material, which should not be misinterpreted as necrosis.

Atypical carcinoids can have larger cells with some nuclear and cellular polymorphism and distinct nucleoli.

Immunophenotype

Carcinoid tumors express the neuroendocrine markers chromogranin, synaptophysin, and CD56. Most carcinoids are negative for TTF1. 20–25% are negative for cytokeratin.

Differential Diagnosis

Neuroendocrine tumors of the lung include typical and atypical carcinoids and the high grade small-cell lung carcinomas (SCLC) and large-cell neuroendocrine carcinomas (LCNEC). Accurate distinction between carcinoid and the high-grade neuroendocrine carcinomas is of greatest importance for appropriate clinical management.

In contrast to carcinoid tumors, SCLC and LCNEC are characterized by a heterogeneous cell population with a high mitotic rate, frequently with large areas of necrosis and nuclear and cellular polymorphism. Compared to the finely and evenly dispersed chromatin of SCLC, carcinoid tumors have a more granular chromatin. LCNEC have cell characteristics of non-small-cell carcinomas and show coarse or vesicular chromatin with prominent nucleoli in large polymorphic cells.

Carcinoid tumors can exhibit pronounced crush artifacts and nuclear molding, which should not be misinterpreted as SCLC. The mitotic activity is often less conspicuous in cytological specimens compared to histology. Immunocytochemistry for Ki-67 (MIB-1) can be very useful in difficult cases. The Ki-67 labeling index is low to moderate (<20%) in carcinoid tumors and high (>50%) in high-grade neuroendocrine carcinomas.

Other neoplasms with a monotonous cell population, including lymphomas (when stripped nuclei are present) and well-differentiated adenocarcinomas, can enter the differential diagnosis.

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Carcinoma ex Pleomorphic Adenoma, Cytological Findings

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Synonyms

Malignant mixed tumor (not appropriate: see differential diagnosis)

Definition

Carcinoma ex pleomorphic adenoma (CEPA) is a carcinoma which arises from epithelial cells in pleomorphic adenoma (Barnes et al. 2005; Ellis and Auclair 2008). Clinically, CEPA presents in two forms: de novo or recurrent with in situ or infiltrative pattern. The clinical data and previous history of salivary (lacrimal) tumor are important in the diagnostic process.

Although CEPA belongs to high-grade malignancy, clinical prognosis depends on histological

type of carcinoma arising in pleomorphic adenoma.

Clinical Features

- **Incidence**
  CEPA is a relatively common malignancy and represent approximately 2% of all salivary gland lesions and more than 6% of salivary gland carcinomas. It develops 12 years in average after a diagnosis of underlying pleomorphic adenoma.
- **Age**
  CEPA occurs at any age, but its peak of incidence is in the sixth and eighth decades of life.
- **Sex**
  There is a slight female-to-male predilection.
- **Site**
  Usually CEPA occurs in the parotid and submandibular glands, major glands being a site of almost 80% of CEPAs. Palate is the most common site in the minor salivary glands.
- **Treatment**
  Treatment for CEPA often involves an ablative surgical procedure which may be followed by radiotherapy.
- **Outcome**
  Recurrences and metastases are common.

Macroscopy

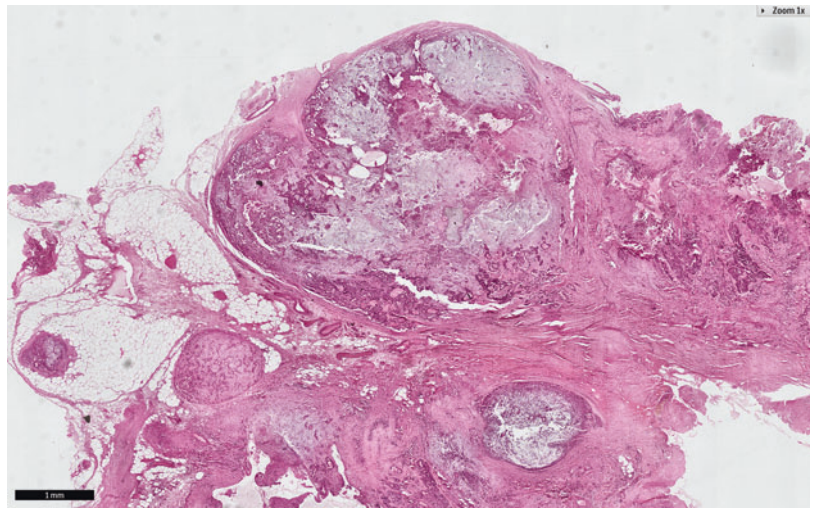
Careful macroscopic examination allows detecting yellowish PA and white-gray component of carcinoma. Tumors may be large in size with necrotic and cystic spaces.

Microscopy

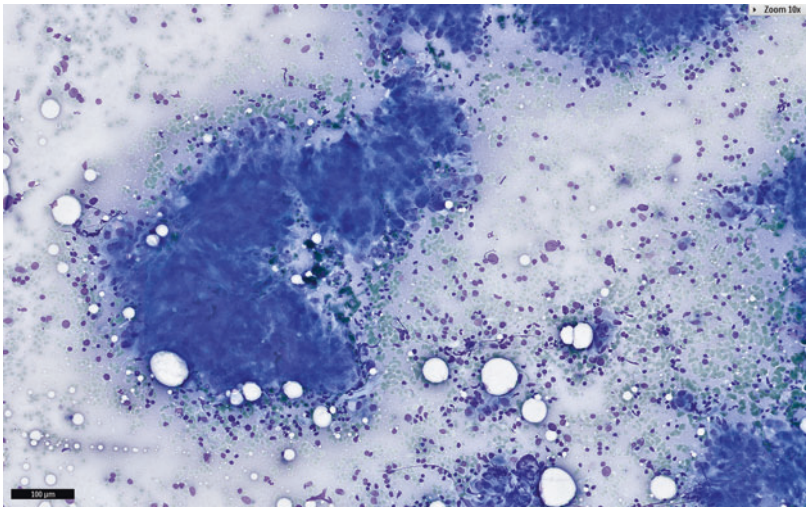
Smears in CEPA are usually hypercellular and cell-rich and stroma-rich. CEPA belongs to the group of tumors exhibiting predominant epithelial cell morphology (Figs. 1 and 2).

Tumors which arise in pleomorphic adenoma are "adenocarcinomas NOS," salivary duct carcinomas, and high-grade mucoepidermoid carcinomas. Tumors like acinic cell carcinomas, polymorphous low-grade carcinomas, and adenoid cystic carcinomas are exceptional (Ersöz et al. 1998; Nigam et al. 2004; Anand and Brockie 1999; Klijanienko et al. 1999a).

It usually is admitted that infiltrative tumors are easier to diagnose than in situ neoplasms and that the overall accurate diagnostic rate may be low. However, high-grade carcinomas are more accurately diagnosed than low-grade carcinomas. It was demonstrated that diagnosis of malignancy is easy in recurrent infiltrative high-



Carcinoma ex Pleomorphic Adenoma, Cytological Findings, Fig. 1 Carcinoma ex pleomorphic adenoma. Multifocal pleomorphic adenoma and carcinoma (*upper-right*) (H&E stain)



Carcinoma ex Pleomorphic Adenoma, Cytological Findings, Fig. 2 Carcinoma ex pleomorphic adenoma. High-grade carcinoma is detected on cytology smears. Histological examination diagnosed salivary duct carcinoma ex pleomorphic adenoma (MGG stain)

grade CEPAs and difficult in de novo in situ low-grade CEPAs. An accurate diagnosis is possible only when patterns of malignancy and of pleomorphic adenoma are encountered in the same slide.

Immunophenotype

There is no utility to use immunohistochemistry.

Molecular Features

CEPA has been extensively studied using molecular techniques. Molecular studies have revealed that the development of CEPA follows a multistep model of carcinogenesis, with the progressive loss of heterozygosity (LOH) at chromosomal arms 8q, then 12q, and finally 17p (Antony et al. 2012). It was demonstrated that (i) alterations at regions on chromosome arms 8q and/or 12q may constitute early events associated with pleomorphic adenomas; (ii) LOH at 12q loci may identify a subset of adenoma with potential progression to carcinoma; (iii) acquisition of additional alterations at chromosome arm 17p loci might represent an event preceding malignant transformation and progression; and (iv) 8q, 12q, and 17p regions may harbor tumor suppressor genes involved in the genesis of PA and CEPA (El-Naggar et al. 2000).

Differential Diagnosis

CEPA should be differentiated from metastasizing pleomorphic adenoma (MPA) and malignant mixed tumor (MMT). Morphologically MPA do not differ from benign PA or benign areas of PA within CEPA, whereas MMT is a collision tumor of carcinoma and sarcoma (carcinosarcoma). The partial diagnosis of "carcinoma" instead of CEPA is sufficient to appropriate patient management (Klijanienko et al. 1998). Finally, some tumors may have synchronous lacrimal and parotid glands presentation and necessitate mutual differential diagnosis (Klijanienko et al. 1999b).

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Cat-Scratch Disease, Cytological Findings

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Synonyms

Bartonella hensellae infection; Necrotizing granulomatous lymphadenitis

Definition

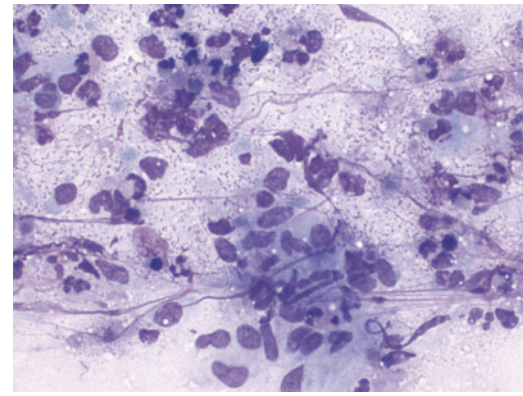
Cat-scratch disease is a bacterial infection, most commonly by *Bartonella hensellae* that clinically manifests with fever, localized lymphadenopathy proximal to the scratch or bite of a cat.

Clinical Features

- **Incidence**
  9/100 000.
- **Age**
  All ages can be infected but mostly patients under 21 years.
- **Sex**
  Equal ratio between males and females.
- **Site**
  Most often the cervical, axillary, epitrochlear, or supraclavicular region is the site of the disease.
- **Treatment**
  Antibiotics have been used and Azithromycin has been suggested as an effective therapy.
- **Outcome**
  In the majority of the cases, the disease has a self-limited course with an excellent prognosis.

Microscopy

In the early phase, the lymph nodes show a mixed lymphoid hyperplasia. Granulomas with histiocytes and multinucleated giant cells are seen in later stages as well as small areas of necrosis (Fig. 1).



Cat-Scratch Disease, Cytological Findings, Fig. 1 Smear from lymph node aspirate shows neutrophils, histiocytes in loose cluster and few lymphocytes. MGG

Immunophenotype

Polytypic (both kappa and lambda) lymphoid cells.

Differential Diagnosis

Other causes of necrotizing granulomatous lymphadenitis, lymphogranuloma venereum.

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Cell Block Techniques

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Alameda Prof. Hernâni Monteiro, Porto, Portugal

Synonyms

Cell block

Definition

Cell-blocks technique is a method through which paraffin embedded blocks from cytological specimen (fine needle aspirates, cutting needle cores, body fluids and residual sediment or other cytological specimen) or from small histological specimens can be obtained/produced.

Principle

Cell block technique is a technique through which liquid cellular samples are turned into a paraffin histologic block.

Methodology

To perform a cell block from a cytologic sample, the aspirate is expelled into a tapered centrifuge tube filled with 10% formalin solution. The syringe and the needle are thoroughly rinsed with formalin to remove the remnants of the aspirate. The tube is centrifuged for 2–3 min at 2,500–3,500 rpm without braking to avoid resuspension of the aspirate. The formalin is decanted and replaced with 0–02% toluidine blue solution in isotonic saline. The tube is centrifuged again and the supernatant decanted.

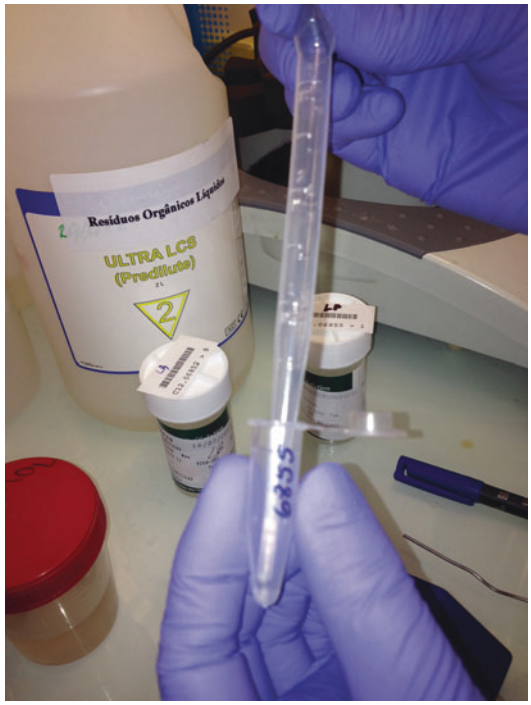
Two drops of plasma are placed on the bottom of the tube, followed by four drops of Simplastin (General Diagnostics; Warner-Lambert Ltd). When clot is formed, the tube is filled with formalin and the clot is handled as a surgical specimen. Another method to perform cell block is the application of "Shandon Cell block method".

Cell blocks can be obtained directly from cell suspensions, fine needle aspirates or cytological fluid material and even from scraped-derived cytological smears.

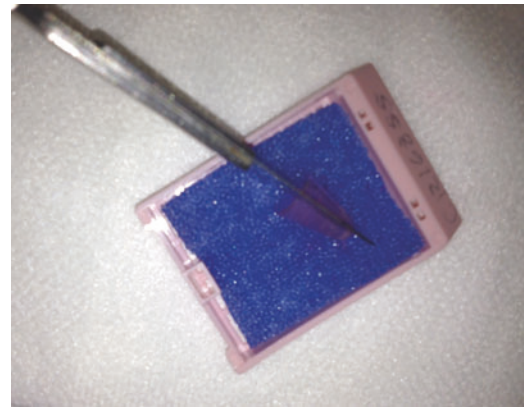
There is a wide variance in the methods that can be used to produce cellblocks which should be mainly defined by: (a) the fixatives used and (b) the fixation time employed, that varies according to the technique (Nathan et al. 2000; Smalley et al. 1992; Olson et al. 1986; Karnauchow and Bonin 1982; Burt et al. 1986).

The choice of reagent used for fixation should depend on the final aim of the end product, which type of cell component(s) to be demonstrated and which histochemical reaction or stain to be selected.

The use of cell blocks for processing cytology fluids is used since 1947 when Chapman and Whalen first described the technique applied on serous fluids (Chapman and Whalen 1947). Many methods have been described since then, with



Cell Block Techniques, Fig. 1 Cell Block technique using Histogel. Step 1 - In this technique, the cell suspension is centrifuged and a pellet is formed. Then the supernatant is removed and the liquefied Histogel is added



Cell Block Techniques, Fig. 2 Cell Block technique using Histogel. Step 2 - After solidification the Histogel with the included sample is removed from the tube and cut into two halves to be included in a tissue cassette, embedded in paraffin and processed as standard histology specimen

a wide range of techniques applied, such as the histogel method (Figs. 1 and 2), gelatine embedding, agar embedding, plasma-thrombin method, or collodion method.

Basically, after fixation of the specimen, the cells should be concentrated by centrifugation and the excess of fluid drained out. The resulted specimen should be mixed with an agglutinant reagent, which can vary accordingly to the technique you are using. This preparation obtained after multiple steps, is included in a paraffin block and handled as a histological sample. All these multiple steps can vary in order of processing cytological material or small biopsies.

Quality Aspects

The main utility of cell blocks is not mainly directed through the possibility of achieving a diagnosis in a material were architecture is more or less preserved, but to enable multiple histological sections for immediate/concomitant or future complementary diagnostic techniques (quantification of mitotic figures, special stains, immunophenotyping, **F**lorescent **I**n Situ **H**ybridization (FISH), **C**hromogenic **I**n Situ **H**ybridization (CISH), and in-situ **P**olymerase **C**hain **R**eaction (PCR)).

Advantages: The origin of the sample used in preparing the cell block does not interfere in the final result and cytological and architectural details are maintained.

The major advantage achieved with this technique is the undoubted large number of slide samples for extensive antibody panels in immunohistochemistry or molecular techniques.

- Numerous sections (10–20 sections) can be made from each sample.
- Storage of cell blocks is much easier than storage of smears.
- Interpretation of immunostains is also much easier based on the preservation of the architecture of your material.
- Preparations are more prone to a clean background in immunostains.

Drawbacks: It is not easy to obtain cell blocks in routine and sometimes material is lost during procedure, for the reason that, processing cell blocks requires a customized treatment and care. Cell blocks should not be processed together with micro biopsies as they contain a more sensible cytological material. The time left in paraffin bath should be shortened to prevent overheat and tissue damage.

The limited quantity of material to perform your cell block might be a further problem. Material obtained from cell suspensions (ex: urine, spinal fluid) or fine needle aspirates can be scarse especially in hypocellular or fibrotic lesions; so that the number of cells obtained are not enough to perform a cell block (e.g., soft tissue tumours). To ensure enough material, a good interaction with radiologist, surgeon or whoever makes fine needle biopsy, should be the first and foremost step in order to collect good material and bet on the right decision of selecting enough material to perform a quality cell block rather than performing an excessive number of smears. For this purpose the on-site assessment of material by a cytopathologist or cytotechnologist might be extremely important even if this requirement is not always easy in a demanding daily routine.

Cell Block Techniques, Table 1 Examples of the application of ancillary techniques in the diagnosis, prognosis and therapeutics of solid tumours, validated for cell blocks

	Cytologic material	Ancillary techniques; clinical significance and important markers
Lung (Delattre C et al. 2011)	EBUS, FNB, washes, serous fluids	Immunostains- diagnosis: SCC/NSCC; ADC/SqCC
		FISH-prognosis and therapeutics: EGFR; ALK
Breast	FNB	Immunostains- Treatment and prognosis Estrogen,progesterone Receptor, HER 2
		CISH: Treatment and prognosis (HER 2)
RMS	FNB	Immunostains- diagnosis
		FISH- diagnosis and prognosis: t(2;13)(q35;q14); t(1;13)(p36;q14)
SS[a] (Taylor CA et al. 2005)	FNB	Immunostains- diagnosis
		FISH-diagnosis and prognosis: t(X;18)(p11;q11); t(X;18)(p11;q11); t(X;18)(p11;q13); t(X;20)(p11;q13)
EWING[a]	FNB	Immunostains- diagnosis
		FISH-diagnosis: t(11;22)(q24;q12)
Liposarcoma	FNB	FISH- diagnosis: Mdm2
Myxoid liposarcoma (Been L et al. 2011)	FNB	FISH-diagnosis: t(12;22)(q13;q12)
DSRCT	FNB	FISH-diagnosis: t(11;22)(p13;q12)
Extraskeletal myxoid chondrosarcoma	FNB	ImImmunostains-diagnosis;
		FISH- Prognosis: t(9;22)(q22;q12); t(9;17)(q22;q11); t(9;15)(q22;q21); t(9;22)(q22;q15)
NB	FNB	FISH-Prognosis and therapeutics: N-Myc; del1p; 17q gains
Renal cell carcinoma	FNB	FISH-diagnosis: t(X;17)(p11;q25)
ASPS	FNB	FISH-diagnosis: t(X;17)(p11;q25)
Congenital fibrosarcoma	FNB	FISH-diagnosis: t(12;15)(p13;q25)
NUT carcinomas	FNB	FISH- diagnosis; t(15;19)

RMS rhabdomyosarcoma, *SS* synovial sarcoma, *FNB* fine needle biopsy, *EBUS* endobronchial ultrasound biopsy, *ADC* adenocarcinoma, *SqCC* squamous cell carcinoma, *SCC* small cell carcinoma, *NSCC* non small cell carcinoma, *DSRCT* desmoplastic small round cell tumour, *NB* neuroblastoma, *RCC* renal cell carcinoma, *ASPS* Alveolar soft part sarcoma
[a]Kumagai A et al. 2010

Some antigens epitopes, such as some **C**luster **D**ifferentiation (CD) markers, **C**arcinoembryonic **A**ntigen (CEA), **E**pithelial **M**embrane **A**ntigen (EMA), **C**ytokeratin (CK), **H**uman **E**pidermal **G**rowth **F**actor 2 (HER2/neu), S100 protein, and oestrogens receptors, may be lost or rendered inactive by alcohol-/methanol-based fixatives, and on subsequently processed as cellblocks, consequently immunohistochemical technique should be validated for each antibody. Furthermore the use of a correct control is essential with a positive cell block containing known positive cells that have been fixed and processed using the same reagents and technique as the test sample.

Applications

Cell blocks are adequate and might represent a good choice for ancillary studies such as immunocytochemistry, fluorescent/chromogenic in-situ hybridization tests (FISH/CISH) and in-situ PCR.

Examples

See Table 1 Examples of the application of ancillary techniques in the diagnosis, prognosis and therapeutics of solid tumours, validated for cell blocks.

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Cerebrospinal Fluid, Cytology of Hematopoietic Malignancies

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Synonyms

Leukemia; Lymphoma infiltration

Definition

The involvement of the CNS by leukemia or lymphoma is usually secondary.

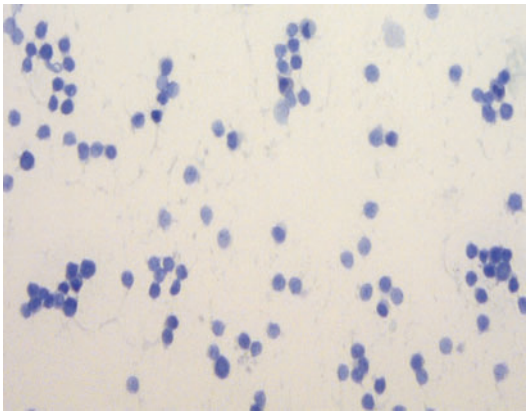
Clinical Features

- **Incidence**
  CNS involvement by leukemia is around 60% in untreated cases, especially acute lymphoblastic leukemia (ALL).

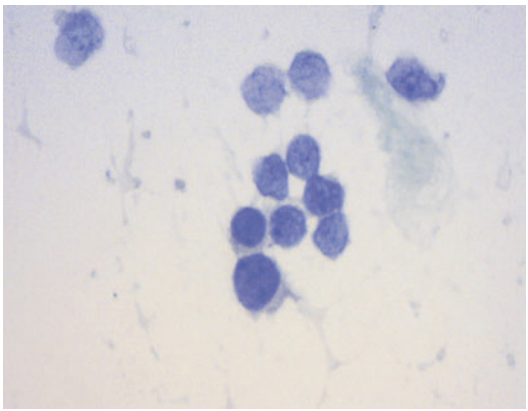
- **Age**
  Involvement by age depends on the injury that affects the CNS. In children is common involvement by ALL and lymphoblastic lymphoma and small non-cleaved cell (Burkitt and non-Burkitt).
- **Treatment**
  The treatment involves radiotherapy and chemotherapy.

Microscopy

Involvement by these lesions is relatively common in nervous system. The cell type is usually difficult to identify. The diagnoses should be established based on clinical data and previous information about the primary disease (bone marrow, lymph node biopsy). The cytologist must identify whether or not CSF blasts. Preparations containing isolated cells, with monotonous aspect and increased in number (Figs. 1 and 2). Giemsa staining helps identify the lymphoid population. Nucleoli in lymphocytes and monocytes usually appear after radiation or chemotherapy.



Cerebrospinal Fluid, Cytology of Hematopoietic Malignancies, Fig. 1 CSF in leukemia – note high cellularity composed of dispersed monomorphic cells (Papanicolaou stain)



Cerebrospinal Fluid, Cytology of Hematopoietic Malignancies, Fig. 2 CSF leukemia-dispersed cells with monotonous aspects (Papanicolaou stain)

Immunophenotype

Flow cytometry can be very useful for imunophenotyping and confirmation of the involvement.

Molecular Features

See chapter on lymphomas and leukemias.

Differential Diagnosis

The most important differential diagnosis includes reactive changes in inflammatory cells usually secondary bleeding or CNS infections. The presence of mixed lymphoid cell population with small cell predominance favors reactive process, and plasma cells are rare in the involvement of CNS by lymphoma/leukemia.

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Cerebrospinal Fluid, Cytology of Infections

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Synonyms

Meningitis

Definition

Inflammatory or infectious process that affects the meninges including among the causes bacterial, virus, fungus, or immunological reactions.

Clinical Features

- **Incidence**
  The incidence varies according to age.
- **Age**
  It affects children and adults and this also means most prevalent etiologic agents in different age groups.
- **Sex**
  Not applicable (N/A)
- **Site**
  Not applicable (N/A)
- **Treatment**
  The treatment involves specific antimicrobial or antibiotic therapy.

Outcome

Bacterial meningitis can be fatal if not treated immediately. Virus meningitis is less fulminate than that of bacterial.

Macroscopy

The CSF may appear whitish, or hemorrhagic xanthomatous, depending on the etiological agent.

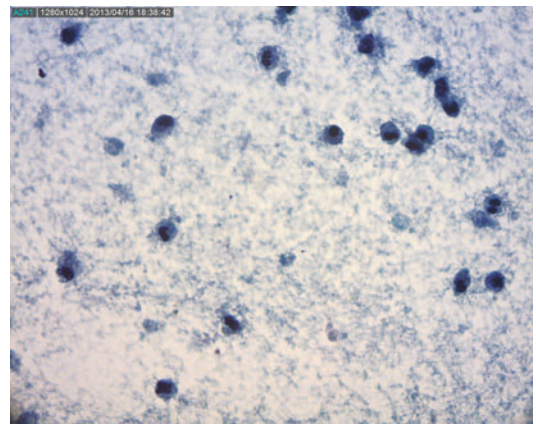
Microscopy

A variety of infectious agents can involve the central nervous system (CSN). The presence of increased number of inflammatory cells in a sample of CSF or atypical lymphoid cells suggests infectious process. The composition of the cellular infiltrate may suggest the etiology of disorders like bacterial, viral, or fungal:

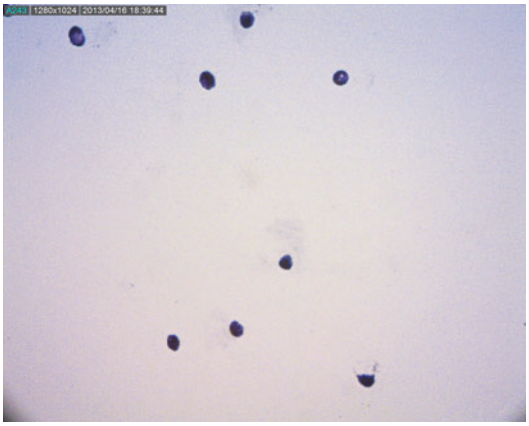
- Neutrophils: bacterial meningitis, viral meningitis, tuberculosis during initial phase
- Lymphocytes: viral meningitis, tuberculosis, bacterial meningitis state later
- Plasma: syphilis, tuberculosis, immune responses
- Eosinophils: parasitic, allergic processes
- Histiocytes tuberculosis, fungal, surgical shunts

Bacterial infections represented by the CSF predominance of polymorphonuclear infiltration, fibrin, macrophages and cell debris can be observed (Fig. 1). The most frequent agents are *Neisseria meningitidis*, *Diplococcus pneumoniae*, *and Haemophilus influenzae*.

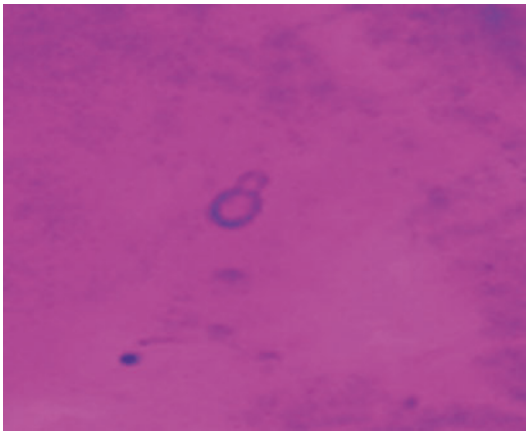
In viral infections, lymphocytes predominate (Fig. 2) and macrophages infiltrate and the causative agents in general include enterovirus groups like coxsackievirus, echovirus, and poliovirus.



Cerebrospinal Fluid, Cytology of Infections, Fig. 1 CSF in acute meningitis – note the high cellularity composed of inflammatory cells, neutrophils, and macrophages (Papanicolaou stain)



Cerebrospinal Fluid, Cytology of Infections, Fig. 2 CSF in viral meningitis – note increased cellularity with a predominance of mature lymphocytes



Cerebrospinal Fluid, Cytology of Infections, Fig. 3 CSF-Cryptococcal organisms with presence of buds (PAS stain)

Cryptococcus neoformans is the most frequent cause of fungal meningitis. Usually affects immunocompromised patients due to hematogenous of disseminating from lung injury. The organisms present round yeast form of variable diameter with thin-necked buds (Fig. 3).

Immunophenotype

Immunocytochemistry can be used to search for specific agents and demonstrate monoclonality in lymphomas or polyclonality in florid pleocytosis as in the cases of Lyme disease.

Differential Diagnosis

The morphological differential diagnosis includes leukemias and lymphomas especially in cases with atypical lymphocytes. However the clinical and biochemical evaluation of the cerebrospinal fluid can help differentiate.

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Cerebrospinal Fluid, Cytology of Metastatic Malignancies

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Synonyms

CNS metastases; Meningeal carcinomatosis

Definition

It is the involvement of the CNS by neoplasms secondarily with discharge cells into subarachnoid space or ventricles.

Clinical Features

- **Incidence**
  Approximately 5% of metastatic tumors in CNS demonstrate meningeal involvement.

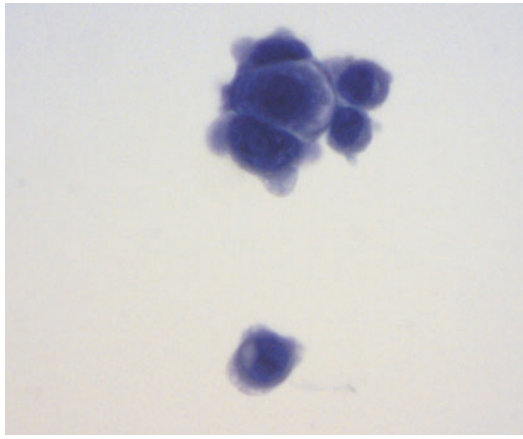
- **Site**
  Fifty percent of metastases are derived from cancers of the lung and breast, 80% of patients have multiple lesions in the brain.
- **Treatment**
  The treatment involves surgery, radiotherapy, and chemotherapy.

Microscopy

In metastasis, CSF presents neoplastic cells with appearance of epithelial cells with atypia. The cells may be single or form small aggregates; the degree of atypia and cell size depends on the primary tumor. Can observe high nuclear: cytoplasmic ratio. The cytoplasm may show little blebs around the margin (Fig. 1). The tumor cells are sometime difficult to distinguish from primary brain tumor, and immunostains are sometime necessary for diagnosis.

Immunophenotype

In metastasis, the positivity for cytokeratin is present in cases of carcinoma; melanoma markers such HMB45 and specific markers for lung and breast cancer may help in specific diagnosis (see entry "Neoplasms of Lung and Breast").



Cerebrospinal Fluid, Cytology of Metastatic Malignancies, Fig. 1 CSF metastasis from breast cancer – few atypical cells grouped, pleomorphic nuclei and coarse chromatin. Cytoplasm show little blebs around the margin (Papanicolaou stain) (Note: Source of the figures of the authors)

Differential Diagnosis

The main differential diagnosis is primary CNS neoplasias. Glial cells can have a look epithelioid, ovoid, or round nuclei delicate chromatin in low degrees and in high-grade nuclear pleomorphism is evident, coarse chromatin and opaque cytoplasm. Glial tumor demonstrates positivity to GFAP (glial fibrillary acidic protein) in immunocytochemistry.

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Cerebrospinal Fluid, Cytology of SNC Tumors

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Definition

Primary neoplasms originate in glial or neuroectodermal cells of the nervous system.

Clinical Features

- **Incidence**
  Not applicable (N/A) – see CNS neoplasias.
- **Age**
  Not applicable (N/A) – see CNS neoplasias.
- **Site**
  Not applicable (N/A) – see CNS neoplasias.
- **Treatment**
  The treatment involves surgery, radiotherapy, and chemotherapy.
- **Outcome**
  Not applicable (N/A) – see CNS neoplasias.

Macroscopy

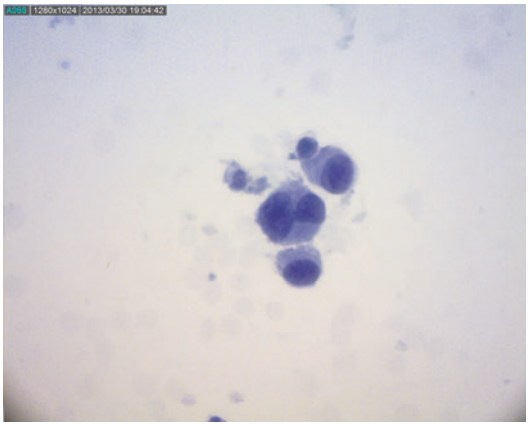
Not applicable (N/A) – see CNS neoplasias.

Microscopy

Gliomas (astrocytomas) are the most frequent primary neoplasms in adults and in children. A minority of gliomas shed diagnostic cells in CSF generally high-grade or advanced tumors.

This presentation depends on the degree of cell injury. Cells look epithelioid, ovoid, or round nuclei delicate chromatin in low degrees and in high-grade nuclear pleomorphism is evident, coarse chromatin and opaque cytoplasm, distinct edges.

- Ependymoma is the most common tumor in the spinal cord columnar epithelial cells aspect, presents fine chromatin and inconspicuous nucleoli, rosettes can be observed.
- Oligodendroglioma presents when detect in liquor monotonous cells, delicate cytoplasm, round nucleus, clear, finely granular chromatin.
- Tumors of neural crest – medulloblastoma, neuroblastoma, and retinoblastoma – occur in children and young adults. It is not possible to differentiate morphologically these three entities, and attention is necessary to differentiate the groups of leukemias. They present small cells, hyperchromatic nucleus, scant cytoplasm, nuclear molding, hyperchromatic, rosettes are rare. Around 25% of patients with medulloblastoma have positive CSF.



Cerebrospinal Fluid, Cytology of SNC Tumors, Fig. 1 CSF in germ cell tumor – note single cells with moderate cytoplasm, round nuclei, and prominent nucleoli (Note: Source of the figures of the authors)

Germ cell tumors occur in children and adolescents, are more common in males than females, and occur more often in the pineal areas and suprasellar region. Single cells with moderate cytoplasm, round nuclei, and prominent nucleoli are observed (Fig. 1).

Immunophenotype

Glial tumors demonstrate positivity to GFAP (glial fibrillary acidic protein) in immunocytochemistry. Medulloblastoma expressed positivity for synaptophysin.

Molecular Features

Not applicable (N/A) – see CNS neoplasias.

Differential Diagnosis

The differential diagnosis of these lesions includes metastasis, lymphomas, and leukemias (see CFS in these lesions). Metastasis tends to

shed large atypical cells in more cohesive groups with abundant and dense cytoplasm. Lymphomas/leukemias present isolate lymphoid cells with enlarged nuclei with lobulated or irregular nuclear membranes or protrusions. The chromatin varies from powdery fine to coarse.

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Cerebrospinal Fluid, Normal Cytology

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Definition

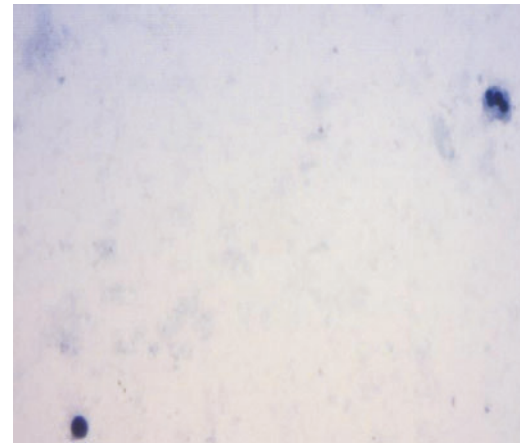
The evaluation of CSF has diagnostic purposes in inflammatory processes (infectious) and the detection of neoplasias. Produced by the choroid plexus in the ventricles via ultrafiltration of plasma, it is reabsorbed through the villi arachnoid being produced about 500 ml/24 h. Leptomeningeal and ependymal cells are rarely observed in its analysis uses the cytological aspects found the patient's age, medical history, and results of laboratory tests of interest. Most samples are originate from lumbar puncture, but can be obtained from the ventricles. It should indicate the location of the sample, because the cellular components are distinct The prepared material must be obtained through concentration method. It is recommended that preparations be stained by Papanicolaou and Giemsa. The sensitivity of CSF for detecting malignance is about 60%, the specificity is high, and false-positive diagnoses are around 2–3%.

Clinical Features

- **Incidence**
  Over 90% of CSF cytology are negative for malignant cells.
- **Site**
  Most samples are originate from lumbar puncture, but can be obtained from the ventricles. It should indicate the location of the sample, because the cellular components are distinct. The prepared material must be obtained through concentration method. It is recommended that preparations be stained by Papanicolaou and Giemsa.

Microscopy

Generally composed of a few cells represented by lymphocytes (Fig. 1) and monocytes. It is



Cerebrospinal Fluid, Normal Cytology, Fig. 1 Note low cellularity with rare leukocytes (Papanicolaou stain)

rare in leptomeningeal cells and ependymal cells.

Differential Diagnosis

In reactive conditions: (after manipulation of the CNS), CSF demonstrate increase above normal cells – monocytes and cells of the leptomeninges. Ependymal cells, Neurons, Macrophages containing phagocytosed various materials.

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Cholangiocarcinoma, Cytological Findings

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Synonyms

Biliary tract carcinoma; Klatskin tumor

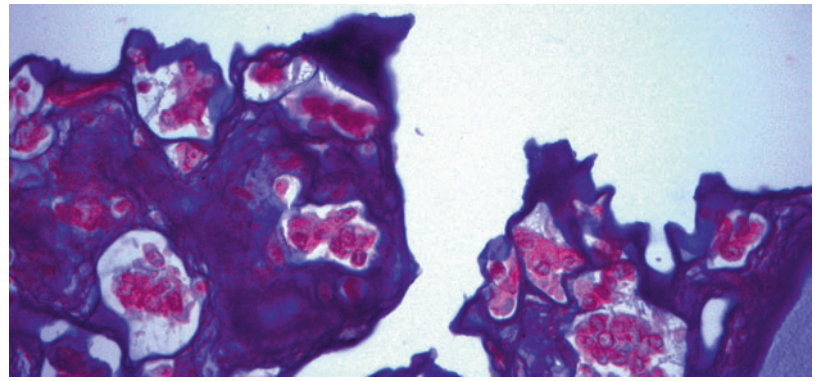
Definition

Adenocarcinomas of the biliary epithelium, of either intrahepatic or extrahepatic biliary tree are called cholangiocarcinomas.

Clinical Features

- **Incidence**
  Cholangiocarcinoma is the second most common hepatobiliary tumor and incidence is rising worldwide. It occurs in approximately 2 per 100,000 people and accounts for approximately 13% of primary liver cancers (Patel 2002). Chronic biliary tract inflammation represents a major risk factor for the development of cholangiocarcinoma (e.g., chronic parasitic infection of the biliary tract, primary sclerosing cholangitis).

  There has been a marked global increase in mortality from intrahepatic, but not extrahepatic, biliary tract malignancies.
- **Age and Sex**
  Age is a risk factor for the development of malignancy and mortality is higher in males in European countries but not in eastern countries.
- **Site**
  Depending on anatomic location, they are classified as: (1) intrahepatic cholangiocarcinoma, occurring in the bile ducts residing within the liver; (2) hilar cholangiocarcinoma, occurring at the confluence of the right and left hepatic ducts; and (3) distal extrahepatic bile duct cancers (Malhi and Gores 2006). Bismuth-Corlette classification is used for hilar lesions as it correlates anatomy with resectability.
- **Treatment**
  The only effective treatment is surgical resection. Neo-adjuvant and adjuvant therapy is being used to attempt to prolong disease-free survival.
- **Outcome**
  The natural history of extrahepatic cholangiocarcinoma is short and median survival ranges in months. Five year patient survival ranges from approximately 20–43%, after surgery in selected patients The best predictors of survival are the absence of lymph node involvement, negative tumor margins up to 1 cm, solitary lesions, and lack of microscopic vascular invasion (Malhi and Gores 2006).



Cholangiocarcinoma, Cytological Findings, Fig. 1 Cell-block of the same lesion from Fig. 2 and Fig. 3 (H&E) Note papillary clusters of malignant cells

Macroscopy

Morphologically, they are classified into mass-forming, periductal-infiltrating, or intraductal-growing.

Microscopy

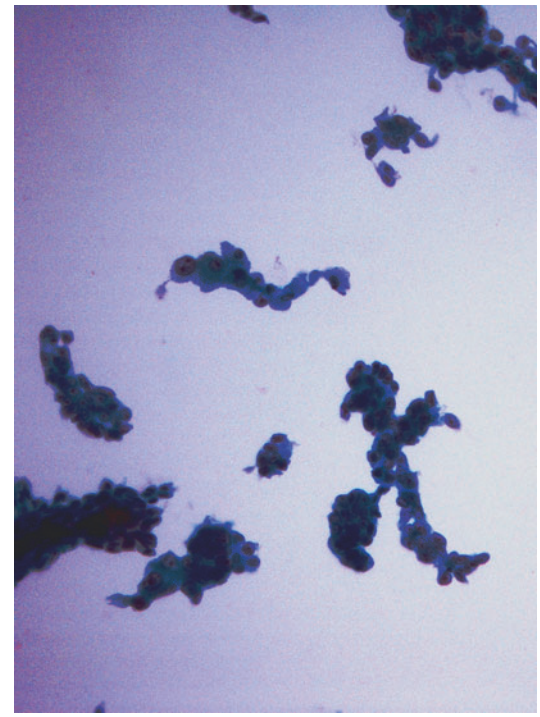
Approach to liver nodules suspected of malignancy is usually sampled for cytological diagnosis by transabdominal FNA (fine needle aspiration) or EUS-FNA (endoscopic ultrasound fine needle aspiration) if their location is in the left lobe (▶ Fine Needle Aspiration Cytology).

Cholangiocarcinomas of extrahepatic bile ducts, if they are not mass-forming, most probably will be sampled by brush cytology during Endoscopic Retrograde Cholangiopancreatography (ERCP) (▶ Biliary Duct Brushing).

Both ways we may have smears, liquid-based cytology (LBC), and cell-block (Fig. 1), but cell content is different.

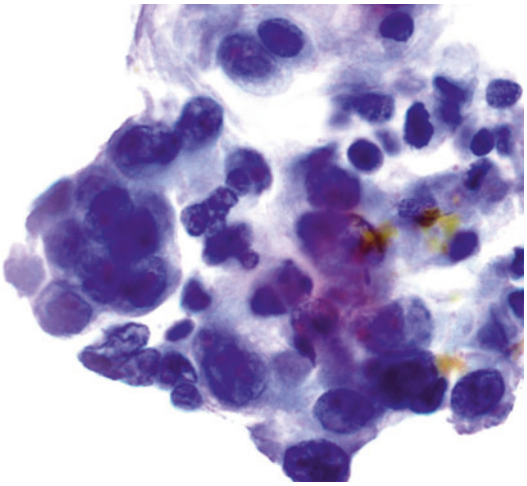
Brush cytology samples represent the whole content of ducts so they will have: cellular debris, biliary pigment, and crystals along with epithelial disorganized clusters, single cells, and small acinar clusters (Fig. 2); groups of cells are three dimensional with a high N:C ratio, coarse chromatin, and prominent nucleoli (Fig. 3).

FNA samples will contain contaminants from the organs adjacent when using EUS-FNA which can be a pitfall on interpreting smears.

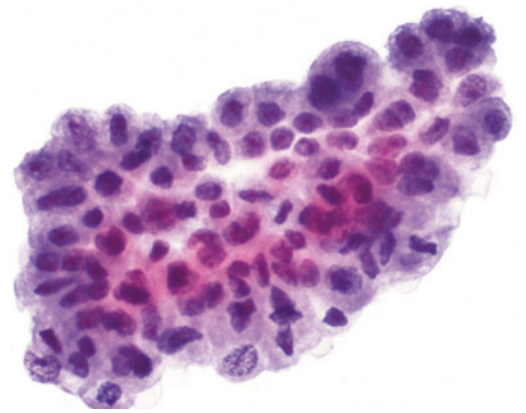


Cholangiocarcinoma, Cytological Findings, Fig. 2 Low power view of small acinar clusters and three dimensional groups of cells - LBC (Pap stain)

Intrahepatic cholangiocarcinomas will show glandular cells in flat angulated sheets (Fig. 4). Low-grade tumors will have nuclear overlapping, irregular nuclear membrane, and chromatin clearing (Fig. 5). Atypia may range from markedly malignant cells to border-line appearance. Stroma also can be seen (Fig. 6),



Cholangiocarcinoma, Cytological Findings, Fig. 3 High power view of a disorganized cluster. Epithelial cells have high N:C ratio, coarse chromatin and prominent nucleoli; note biliary pigment – Pap stain

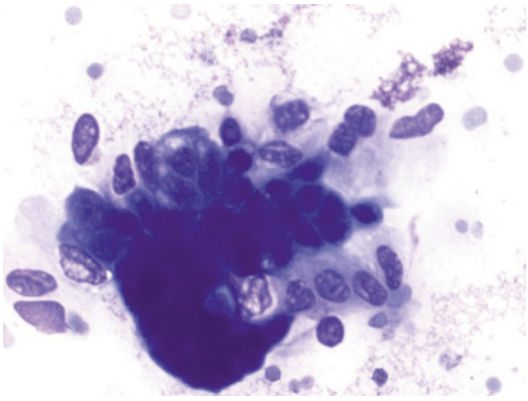


Cholangiocarcinoma, Cytological Findings, Fig. 4 Flat angulated sheet of cell with some nuclear overlapping (Pap stain)

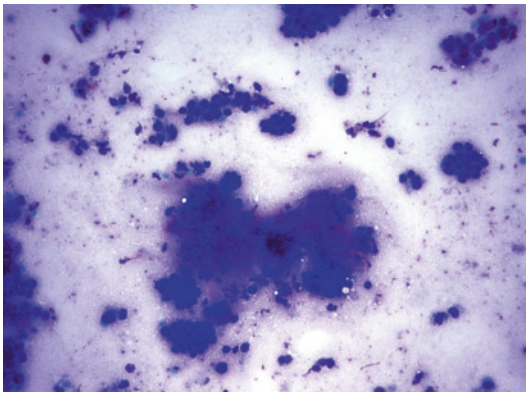
and mucin (Fig. 7) can be seen at least focally (Pitman 2010 and Kocjan 2010).

Immunophenotype

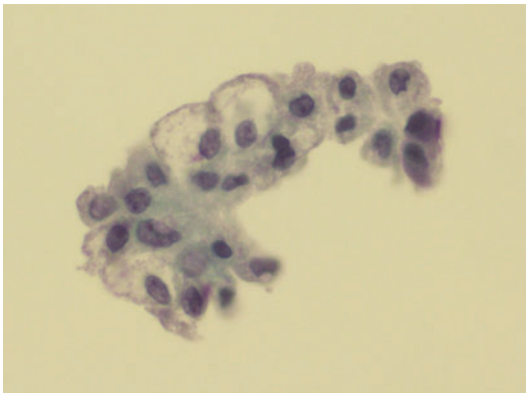
Cytokeratins (CK) 7 and 19 are positive and CK20 is negative. Polyclonal CEA marks diffusely the cytoplasm. LeuM1and B72.3 are positive.



Cholangiocarcinoma, Cytological Findings, Fig. 5 Malignant cells with chromatin clearing and nuclear overlapping (MGG stain)



Cholangiocarcinoma, Cytological Findings, Fig. 6 Fragments of stroma in a papillary tumor (MGG stain)



Cholangiocarcinoma, Cytological Findings, Fig. 7 Mucin-producing cells (Pap stain)

Differential Diagnosis

See "▶ Hepatocellular Carcinoma, Cytological Findings".

See "▶ Liver Metastasis, Cytological Findings".

Differential diagnosis on brush cytology is with benign lesions like adenomas and atypia from inflammatory strictures, which can mimic malignancy on cytology. Moreover, malignancy can arise in a set of inflammation.

Malignant intrahepatic tumors, either primitive like hepatocarcinoma, namely, with an acinar pattern and metastatic adenocarcinomas, are difficult to differentiate.

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Chordomas, Cytological Findings

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Synonyms

Malignant notochordal tumor

Definition

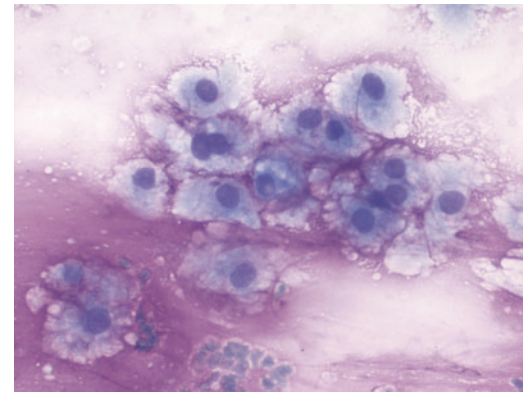
Chordoma is a low-grade malignant tumor that is thought to arise from the remnants of the notochord.

Clinical Features

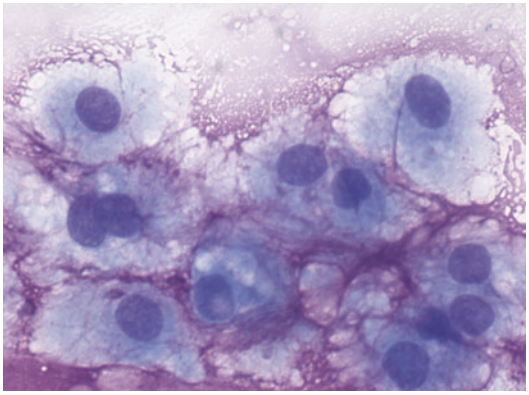
- **Incidence**
  Approximately 4% of malignant bone tumors.
- **Age**
  Predominately adults between 40 and 60 years of age.
- **Sex**
  Male to female ratio 2:1.
- **Site**
  Sacrococcygeal region in 50%, sphenoid in 37%, and vertebras in the remaining cases.
- **Treatment**
  Radical resection is the treatment of choice for the patients with spinal chordoma. In localizations where surgery is difficult, the treatment of choice is radiation therapy.
- **Outcome**
  Low-grade tumor and the morbidity and mortality are related to local progression of the disease. Distant metastasis occurs in 20–30% of cases, usually in the lungs.

Microscopy: Cytological Findings

Fine needle aspiration cytology presents large mononucleated or binucleated tumor cells with bubbly abundant cytoplasm (physaliferous cells), rounded uniform cells, and plump spindle cells in abundant background myxoid matrix (Figs. 1, 2).



Chordomas, Cytological Findings, Fig. 1 Chordoma: tumor cells with round, monomorphic nuclei and rich cytoplasm with large vacuoles (physaliferous cells). *MGG* myxoid background matrix



Chordomas, Cytological Findings, Fig. 2 Chordoma: higher magnification of cells seen in Fig. 1. MGG

Immunophenotype

Tumor cells are positive for cytokeratin, EMA, and S100.

Molecular Features

No tumor-specific rearrangements were found. Hypodiploid or near-diploid and abnormalities of chromosomes 1 and 3 were prominent.

Differential Diagnosis

Metastasis of carcinoma, chondrosarcoma, and meningiomas with myxoid matrix.

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Chromophobe Renal Cell Carcinoma, Cytological Findings

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C

Synonyms

Chromophobic renal cell carcinoma

Definition

The meaning of the term chromophobe carcinoma is that of a tumor that has phobia of color (chromo). When stained with the normal dyes (hematoxylin/eosin), it does not stain readily and thus appears paler under the microscope. Chromophobe renal carcinoma is a rare subtype of Renal cell carcinoma (RCC) with a better prognosis than clear cell carcinoma with many similarities to the intercalated cells of the collecting ducts, which seem to be the cell origin.

Clinical Features

- **Incidence**
  Chromophobe renal carcinoma accounts for 4–6% of all renal carcinomas.
- **Age**
  It affects mainly patients in the sixth decade of life. Presentation in tuberous sclerosis or in Birt-Hogg-Dubé Syndrome clinical set occurs earlier, affecting younger patients.
- **Sex**
  Incidence of chromophobe renal cell carcinoma is similar in both men and women.
- **Site**
  Kidney.
- **Treatment**
  Partial nephrectomy is the usual procedure. Overexpression of CD117 on cellular membranes of chromophobe renal cell carcinoma opens new perspectives in the field of potential

target therapies for kinase inhibitors like imatinib, dasatinib, and nilotinib.

- **Outcome**
  Most cases are diagnosed in stage I and II, and prognosis is better than in other types of RCC. Five- and ten-year disease-free survival is 83.9% and 77.9%, respectively (Amin et al. 2008). Some rare cases of liver metastasis have, however, been described (Stec et al. 2009). Incidence of metastatic disease in chromophobe renal cell carcinoma is 6–7% (Amin et al. 2008).

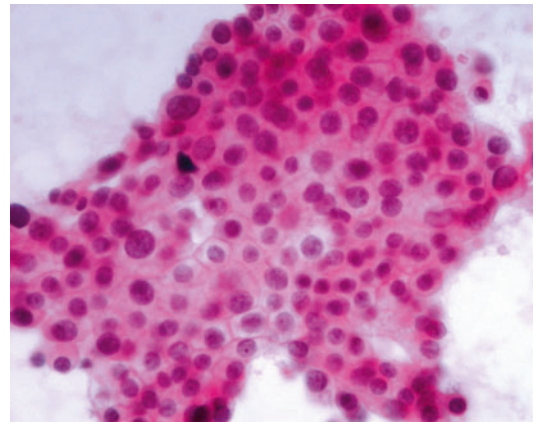
Macroscopy

Chromophobe carcinomas are well circumscribed, with a soft texture, beige or tan. Hemorrhage and necrosis are not present. These tumors are larger than other renal cell carcinomas and have a median size of about 6 cm.

Microscopy

On histology these tumors show a nesting pattern, composed of large polygonal cells with distinct cell border ("vegetable cells" aspect, due to cytoplasmic retraction). Cytoplasm is abundant with reticular pattern (light blue/pale, flocculent, not clear). Some cells have a perinuclear halo or translucent zone ("fried-egg" or koilocytic appearance) (Fig. 1), while other cells show a slightly granular, eosinophilic cytoplasm. Nuclei are hyperchromatic and variable in size and shape. Irregularities of the nuclear membrane can be prominent, giving a typical "raisinoid" appearance. Nucleoli are small or indistinct (Beata et al. 1999; Tejerina et al. 2009; Granter and Renshaw 1997; Geisinger et al. 2004). Mitotic figures can be present but may be scant. Classic type is more aggressive and can show necrosis.

The main diagnostic criteria of chromophobe RCC is morphology coupled with immunohistochemical characteristics: Hale's colloidal iron, diffuse cytokeratin CK7, and **c**luster **d**ifferentiation antibody CD117 positivity.

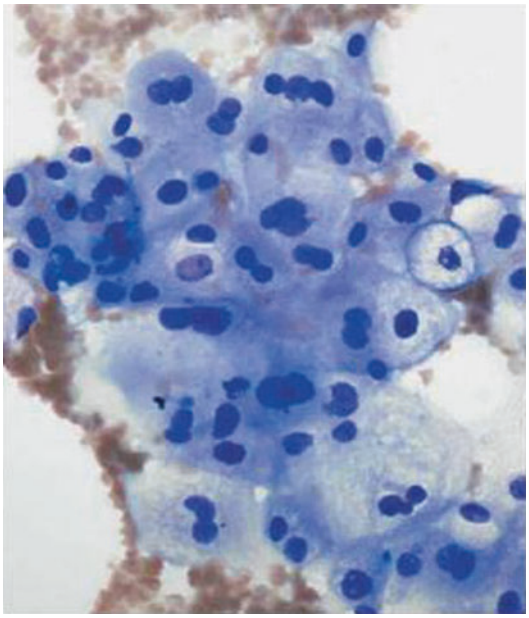


Chromophobe Renal Cell Carcinoma, Cytological Findings, Fig. 1 Chromophobe carcinoma (H&E, 100×) – round polygonal cells with a vegetable aspect. In the center of the cluster, a cell with a perinuclear halo is seen (fried-egg type or koilocytic type). Anisokaryosis is remarkable but nucleoli are small

In cytology, aspirates are generally cellular with large polygonal cells that lay single or in small clusters in a clean background. Cells appear less cohesive than in clear cell carcinoma. Binucleation is frequent and nuclei are large hyperchromatic with variable nuclear sizes and irregularities in the membrane contour. Nucleoli are inconspicuous. Cytoplasm has sharply defined borders (Figs. 1 and 2), vegetable-like appearance, and may exhibit varying degrees of fluffiness or granularity. This lack of uniform aspect, even in the eosinophilic type, helps in the differential diagnosis with other granular renal tumors like oncocytoma. Like in histology the typical aspect of "fried-egg" or "koilocytic" appearance is noticeable. Cytoplasm is not as fragile as in clear cell carcinoma and so nude nuclei are not a common feature. In the eosinophilic variant most cells show a prominent granularity of their cytoplasm.

Immunophenotype

Classically these tumors express diffuse cytoplasmic positivity for Hale's colloidal iron. Hale's colloidal iron positivity can also be seen in oncocytoma (see Section "Differential Diagnosis").



Chromophobe Renal Cell Carcinoma, Cytological Findings, Fig. 2 Fine-needle aspirate of a chromophobe carcinoma. Cells in clusters exhibit a vegetable appearance with cytoplasmic well-defined borders and a fluffy aspect. In the right two koilocytic-type cells can be seen (Giemsa, 200×)

Similarly to liver, adrenal, and parathyroid, the kidney cells tend to bind in a nonspecific way to avidin and peroxidase. These cells have in their cytoplasm endogenous biotin or biotin-binding proteins. This effect may yield a stained background and false-positive staining. Interpretation of immunostains should be cautious.

Chromophobe cell carcinomas stain uniformly to cytokeratin 7 and 18 and variably to cytokeratin 8, 19, and 20. Epithelial membrane antigen (EMA) and parvalbumin are also positive in most cases. Immunoexpression of vimentin and CD10 are variable (Tejerina et al. 2009; Granter and Renshaw 1997; Geisinger et al. 2004).

Molecular Features

Most of chromophobe renal cell carcinomas are sporadic, but sometimes they are associated with Birt-Hogg-Dubé syndrome or appear in a tuberous sclerosis clinical setting.

Chromophobe renal cell carcinoma is characterized by loss of numerous chromosomes including chromosome 1, 2, 3, 6, 7, 9, 10, 11, 12, 13, 17, 18, and 21 (Jones et al. 2005). Loss of 3p as demonstrated in clear cell RCC or trisomy 7 and 17 as demonstrated in papillary RCC has not yet been described in chromophobe renal cell carcinoma, compelling the evidence that chromophobe renal cell carcinoma is a distinct entity.

Differential Diagnosis

Although most authors consider chromophobe renal cell carcinoma an entity with sufficiently distinctive cytological features to permit a correct diagnosis by fine-needle aspiration, several tumors should be considered in the differential diagnosis. Oncocytoma, renal clear cell carcinoma (eosinophilic type), ▶ angiomyolipoma (eosinophilic variant), and papillary carcinoma type II should be excluded.

Cytological criteria are essential in the differential diagnosis with these entities. Immunostains are not specific and are shared with the various entities before mentioned. Even Hale's colloidal iron is not specific.

RCC is characterized by bloody and necrotic smears, and nuclear pleomorphism is generally associated to patent and prominent nucleoli. Nuclei are more regular and uniform than in chromophobe renal cell carcinoma carcinoma. Nucleoli are not prominent in chromophobe carcinoma. Neoplastic cells in clear cell RCC are generally more cohesive, disposed in sheets with a more monotonous cytoplasmic appearance than in chromophobe renal cell carcinoma. In clear cell RCC, cytoplasm is clear sometimes with glycogen or lipidic vacuoles and do not have a fluffy appearance as in chromophobe renal cell carcinoma and are fragile, and so numerous nude nuclei are seen in the background as well as a tigroid appearance (Giemsa stains) due to cytoplasmic disruption with glycogen leakage to the background. Intranuclear pseudoinclusions are not seen in clear cell RCC and favor chromophobe renal cell carcinoma. Hale's colloidal iron is useful and negative in clear cell RCC. Vimentin

is also intensely and diffusely positive in clear cell RCC.

Oncocytoma is another differential diagnosis that must be considered, especially with the eosinophilic variant of chromophobe renal cell carcinoma. Oncocytoma is by definition a low-grade nuclei tumor with no necrosis or mitoses. Nucleoli are small whenever present and nuclei are round and monotonous in shape and size. Appearance is more uniform. In smears, cells are more cohesive in sheets or clusters, and they are large with abundant granular eosinophilic cytoplasm. Cytoplasm is generally preserved, well defined, and no nude nuclei present. Although in some tumors atypia and nucleoli might be prominent, cytoplasm is never fluffy or has clear aspects. Oncocytoma is negative, in most cases, to Hale's colloidal iron and vimentin but stains with low molecular weight keratins (CAM 5.2).

Papillary carcinoma type II has in most smears a papillary architecture. Papillae are covered with large eosinophilic cells. Necrosis and hemorrhage are frequent as well as macrophages in the background. Nuclei grade is high and tumors exhibit a characteristic trisomy 7 and/or 17. Keratin 7 is also positive, although not so constant and intense as in type I. These characteristics help in the differential diagnosis with chromophobe renal cell carcinoma.

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Cirrhosis, Cytological Findings

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Synonyms

Nodular regenerative liver fibrosis

Definition

Diffuse process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules (Anthony et al. 1978).

Clinical Features

Cirrhosis is an important cause of morbidity and mortality. It is found in 4–12% of patients at autopsy in developed countries (Wanless and Crawford 2009). Different types of chronic insult may result in cirrhosis. Alcohol toxicity, chronic viral or autoimmune hepatitis, biliary obstruction, and a variety of metabolic abnormalities are generally implied, and in time result in a morphologic disarrangement, with mechanical, functional, and neoplastic consequences. Cytology is not used for diagnosis. But in this context, nodule may be sampled to exclude neoplasia.

Macroscopy

There are two main forms: micronodular and macronodular cirrhosis. Fibrous bands may be large or thin that ultimate define regenerative nodules. Although a diffuse process, samples from a macronodule may only show normal parenchyma cytology.

Microscopy

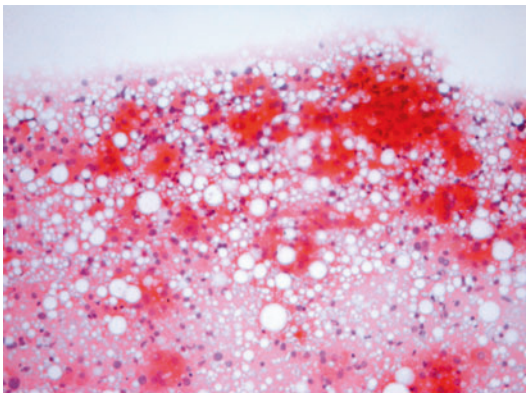
Smears may show clusters of reactive hepatocytes with degenerative/regenerative features, clusters of bile ductal cells, and fragment of fibrous tissue with spindle-shaped nuclei (Mallikarjuna Swamy et al. 2011). If steatosis is a main feature, there will be lipidic vacuoles (Figs. 1–3).

Differential Diagnosis

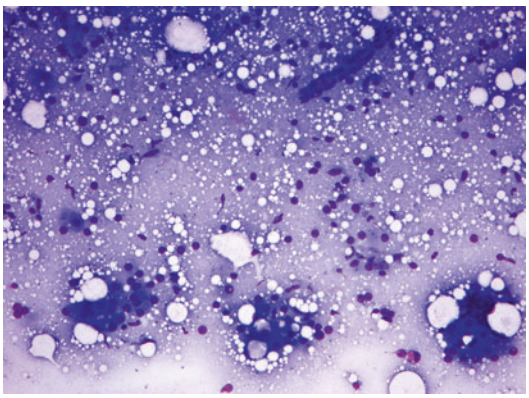
When trying to differentiate benign from malignant hepatocytic lesions, one should evaluate smears at a low-power microscopic view to evaluate the smear pattern. Both benign reactive and neoplastic lesions are variably cellular with benign-appearing hepatocytes in irregularly shaped, jagged-edged clusters without associated peripheral wrapping of endothelial cells (Fig. 4). In addition, transgressing endothelial cells may also be present, but this feature is more common in hepatocellular carcinomas. Nodules of cirrhosis may also produce smooth-edged clusters of cells but without the endothelial cell wrapping. In well-differentiated hepatocellular carcinoma, hepatocytes are monomorphic and small often with macronucleoli.

The presence of reactive and proliferative bile duct cells should alert the pathologist to a benign process rather than a malignant one (Fig. 5).

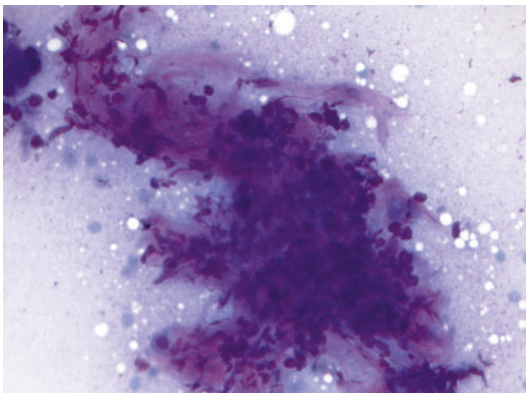
See "▶ Hepatocellular Carcinoma, Cytological Findings".



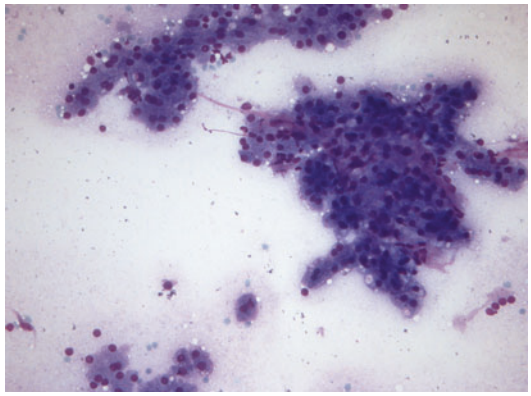
Cirrhosis, Cytological Findings, Fig. 2 Lipid droplets in the background and some groups of benign-appearing hepatocytes (Pap stain)



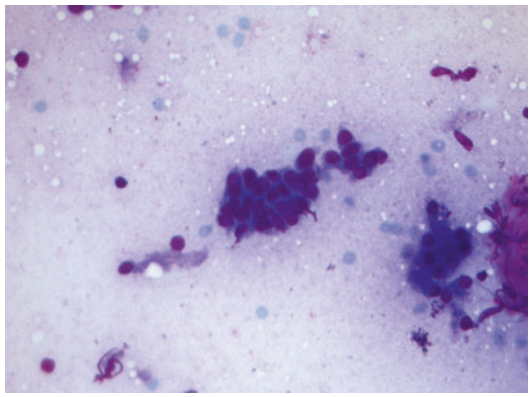
Cirrhosis, Cytological Findings, Fig. 1 Normal components of liver parenchyma: ductal epithelium (*above*) and groups of hepatocytes (*lower field*). Note lipid droplets in the background (MGG stain)



Cirrhosis, Cytological Findings, Fig. 3 Fragment of fibrous tissue (MGG stain)



Cirrhosis, Cytological Findings, Fig. 4 Benign-appearing hepatocytes in irregularly shaped clusters. Note that there are no endothelial cells wrapping the cluster but a transgressing vessel is present (Pap stain)



Cirrhosis, Cytological Findings, Fig. 5 Reactive/proliferative bile duct cells (MGG stain)

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Clear Cell Sarcoma, Cytological Findings

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Synonyms

Malignant melanoma of the soft parts

Definition

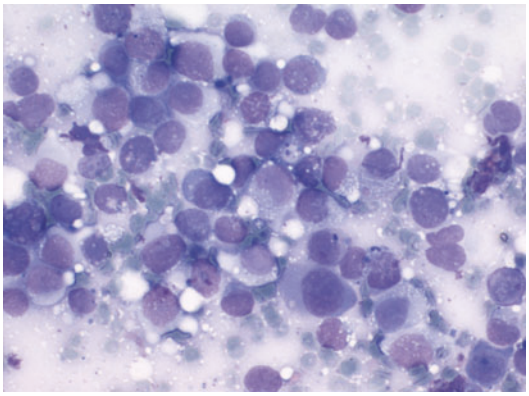
It is a sarcoma of the soft tissue with melanocytic differentiation, typically involving tendons and fascia of distal extremities of young adults. First described by Enzinger in 1965.

Clinical Features

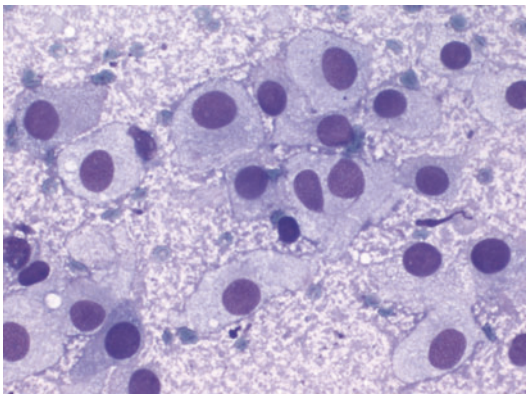
- **Incidence**
  Rare tumors, less than 1% of all soft tissue tumors.
- **Age**
  Young adults, median age 30 years.
- **Sex**
  Slight female predominance.
- **Site**
  It occurs in the tendons and aponeuroses of the distal extremities. Lower extremity is the commonest site (75%).
- **Treatment**
  Primarily by surgical resection.
- **Outcome**
  Clear cell sarcoma of soft parts is a highly malignant tumor with aggressive clinical course and poor prognosis. Often metastasizes to the lymph nodes.

Microscopy

The aspirates are often cellular, consisting predominantly of dispersed cells, but also loose clusters of cells can be found. The cells have abundant polygonal cytoplasms and round nuclei with prominent nucleoli. Intranuclear cytoplasmic inclusions and "tigroid background" have been described. Other features, including spindle cells, clear cells, intracytoplasmic melanin pigment, marked pleomorphism, bi- and multinucleated giant cells, and focal microacinar pattern, occur in variable frequency (Kumar et al. 2003) (Figs. 1 and 2).



Clear Cell Sarcoma, Cytological Findings, Fig. 1 Clear cell sarcoma: polymorphic tumor cells with rounded nuclei and large clear *light grey* distinct cytoplasma (MGG)



Clear Cell Sarcoma, Cytological Findings, Fig. 2 Clear cell sarcoma: dissociated monomorphic cells with round nuclei and large distinct clear cytoplasm (MGG)

Immunophenotype

Tumor cells are positive for S100, HMB45, Melan A, and Vimentin.

Molecular Features

Reciprocal translocation t(12;22)(q13;q12) which results in fusion of *EWS* (22q12), and *ATF1* (12q13)genes is the specific cytogenic and molecular abnormalities.

Differential Diagnosis

Cutaneous melanoma, epithelioid, alveolar soft part sarcoma, synovial sarcoma and metastasis of poorly differentiated carcinomas.

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Clear Cell Tumor of Lung, Cytological Findings

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Synonyms

Benign clear cell tumour; Clear cell tumour; Sugar tumour

Definition

Clear cell tumors or sugar tumors are so named because of their rich glycogen content. This entity is thought to be essentially benign but has an uncertain histogenesis and is probably part of the same family as the PEComas (perivascular epithelioid cell tumors) (Nicholson 2004). Although these tumors have an unknown histogenesis, they have a unique morphological appearance and immunohistochemical properties. As the name "clear cell" would suggest, the cells of this tumor have abundant water-clear, eosinophilic cytoplasm and are glycogen rich. Clear cell tumor was first described by (Leong and Meredith 1997).

Clinical Features

- **Incidence**
  These tumors are very rare and patients are usually asymptomatic. These tumors may present as an incidental finding. Although most clear cell tumors arise de novo, they may occur in associated with tuberous sclerosis and lymphangioleimyomatosis (Armando and Dail 2008).
- **Age**
  There is a wide age range of occurrence ranging from as young as 8 to as old as 73 years with the median age of 40 years old (Nicholson 2004; Leong and Meredith 1997).
- **Sex**
  There is a female predominance.
- **Site**
  Clear cell tumors are classically solitary, localized, and present peripherally in the lungs. It usually presents as a coin lesion (Colby et al. 1991). If these tumors occur outside the lungs, they are known as myomelanocytomas. They are usually within 2 cm of the pleura but usually do not involve the pleura layers, bronchi, or the vessels nor are they associated with a pleural effusion (Nicholson 2004; Leong and Meredith 1997).
- **Treatment**
  Virtually all tumors are cured by complete surgical excision (Nicholson 2004).
- **Outcome**
  The outcome of patients with clear cell tumor of the lung is essentially good as these tumors usually follow as benign course. As previously mentioned, these patients are cured by surgical removal of the tumor. There is only one reported fatal case of a patient dying from metastatic disease, 7 years after initial presentation.

Macroscopy

These tumors may present as a coin lesion radiographically and are usually 20 mm in diameter (size range between 1 mm and 65 mm) (Nicholson 2004; Colby et al. 1991). They are typically well circumscribed, solitary, and unencapsulated, making it readily shelled out from the surrounding lung by the surgeon. The cut surface of the tumor often shows a nodular, red to tan appearance. These tumors do not exhibit necrosis or hemorrhage.

Microscopy

On low-power light microscopy, these tumors exhibit a nodular pattern with delicate vascular pattern around nests of tumor cells. On higher magnification, the tumor cells have distinct cell borders and are generally oval but can be elongated, spindled, or rounded. Occasional large "spider cells" with strands radiating outwards from their granular cytoplasm may be identified. In addition, giant cells are not uncommon. As the name "clear cell tumor" might suggest, the tumor cells have abundant clear, eosinophilic cytoplasm. These exhibit strong diastase-sensitive PAS positivity (Leong and Meredith 1997). They generally lack cytological atypia and mitoses are generally absent. There may be a range of nuclear appearances ranging from small, dense chromatin to large vesicular nuclei with distinct nuclear membrane and prominent nucleoli. Necrosis is rare seen. One should consider a malignant tumor if there is significant mitotic activity, if the tumor is infiltrative, or if there is evidence of necrosis. Associated with the nests of tumor cells are prominent, ectatic, thin-walled sinusoidal vessels which may occasionally be hyalinized but generally do not have a muscular coat (Nicholson 2004). Ultrastructurally, these tumors contain melanosomes and have abundant free and membrane-bound glycogen (Armando and Dail 2008).

Immunophenotype

These tumors are positive with melanocytic markers, namely, HMB45 and S100 but are negative with cytokeratin markers (Nicholson 2004; Armando and Dail 2008). Neuroendocrine differentiation may be demonstrated immunohistochemically, but the histogenesis of these tumors is uncertain (Colby et al. 1991).

Molecular Features

There are no described molecular features

Differential Diagnosis

The following are the differential diagnosis of a clear cell tumor (Nicholson 2004; Armando and Dail 2008):

1. Clear cell carcinoma (primary or metastatic)
2. Metastatic renal cell carcinoma
3. Granular cell tumor
4. Metastatic melanoma
5. Metastatic clear cell sarcoma

In addition, entities such as sclerosing hemangioma, epithelioid hemangioendothelioma, and carcinoid tumor may all have some clear cell features (Colby et al. 1991).

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Colloid Goiter, Cytological Findings

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Synonyms

Multinodular goiter; Nodular goiter

Definition

The term goiter refers to any enlargement of the thyroid gland and is considered as a nonneoplastic process, usually benign, essentially due to iodine deficiency, but sometimes of unknown etiology. The term goiter includes both either a thyroid gland deformed by numerous nodules of varying sizes or a diffuse enlargement of the gland. The nodular goiters are histologically represented by two different types of nodules: the **colloid nodules** related with the accumulation of the colloid within the follicles and the **hyperplastic (adenomatoid) nodules** characterized by a hyperplasia of the follicular cells.

Clinical Features

- **Incidence**
  Nodular goiters represent one of the most frequent diseases in the world with prevalence from 1% to 10% depending on geographic locations. Goiters are more frequent in mountains than in the low lands and on the seaside. In the United States, there is an estimated annual incidence rate of 0.1% per year.
- **Age**
  The goiter increases with age.
- **Sex**
  It is more frequent in women.
- **Site**
  Goiter is a term which means that the gland is entirely modified.
- **Treatment**
  Treatment depends on the clinical data; in case of iodine deficiency, iodine-rich foods may be introduced into the diet, but it is often not sufficient. Hormone substitutive therapy is required in case of hypothyroidism. In case of hyperthyroidism drugs blocking the gland are necessary. Goiter without any hormonal trouble may benefit either from surgery or from radioiodine therapy when the nodules are voluminous and troublesome, eventually compressive. Mostly, considering that nodular goiter is a nonneoplastic disease, it does not require surgery.
- **Outcome**
  Usually excellent since it is a benign disease. Extreme hyper- or hypothyroidisms forcing immediate treatment are rare. On the counterpart there is about a 5–7% risk of cancer. Depending on the studies this risk is considered lower as the risk of cancer in a single nodule or equal.

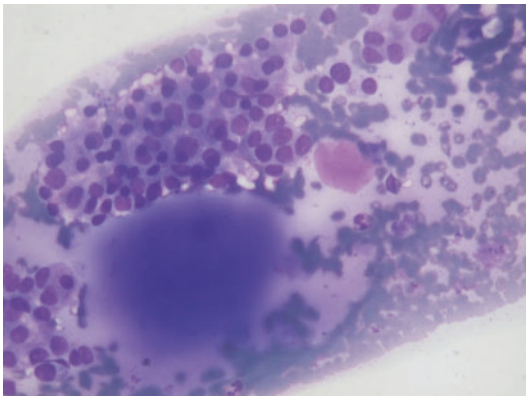
Macroscopy

Lobectomy as well as near total thyroidectomy may be examined. The number of nodules varies a lot from 3 to 5 nodules per lobe to more than 20 nodules. The nodules vary also in size from 4–5 mm to 3 cm or more; finally, the nodules show different aspects depending on the quantity of colloid and the existence or not of hemorrhagic areas.

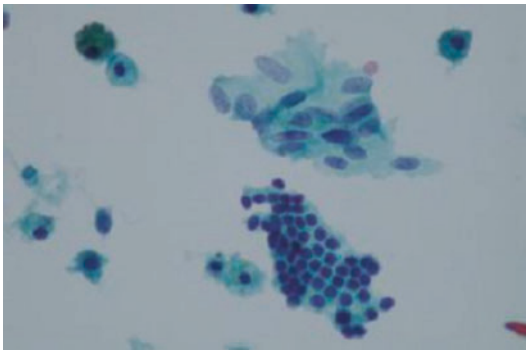
Microscopy

In this chapter, description concerns only the colloid nodules; the hyperplastic nodules are described in another one. Colloid nodules are made up of (1) some monolayered sheets of follicular cells mixed with some sheets of cells with slight nuclear overlapping, (2) abundant colloid, (3) histiocytes with or without hemosiderin, and (4) sometimes spindle-shaped cells with enlarged, elongated nuclei.

The follicular cells include round or oval nuclei with regular borders; there are no microfollicles or really only very few; colloid is clearly seen on the background either as a light blue-pink watery colloid or as a dark blue-purple dense colloid on MGG staining (Fig. 1); watery colloid appears less obviously on Papanicolaou staining, as blue green droplets, sometimes as amphophilic drops when it is dense. For the Bethesda System for Reporting Thyroid Cytology (BSRTC-2009), follicular cells are required to consider the FNA as a diagnostic except when there are really large drops of colloid in great quantities; it is then recognized as a benign colloid nodule.



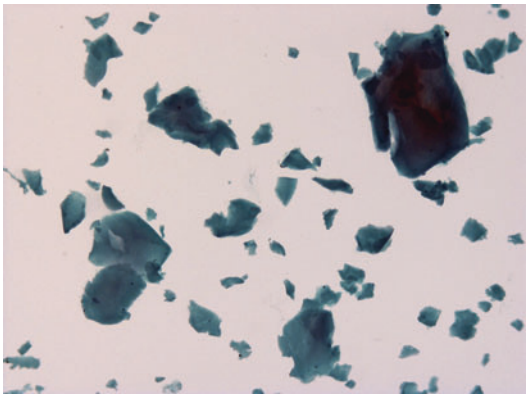
Colloid Goiter, Cytological Findings, Fig. 1 Sheet of follicular cells with regular and round nuclei close to a large drop of blue, dense colloid (conventional slide; MGG ×25)



Colloid Goiter, Cytological Findings, Fig. 2 Monolayered follicular cells with round and hyperchromatic nuclei with spindle-large cells including enlarged nuclei with clear chromatin. These cells are typical cyst-lining cells (LBC Hologic®, Papanicolaou staining ×40)

The spindle-shaped cells with elongated, enlarged nuclei, finely granular chromatin and marked nuclear membrane are also observed in cases with a cystic component; they are called the cyst-lining cells in BSRTC.

In liquid-based cytology, essentially with the Hologic® technique, there are some significant changes mainly due to the alcoholic fixative: (1) the colloid appears either like dense dark blue droplets or like dark blue-orange in the center droplets (Fig. 3); but often, due to the technique, the colloid may be absent; (2) follicular cells are arranged quite exclusively in monolayer sheets. The groups of cells are usually composed of less than 20–25 cells. The cytoplasm is scant, blue pale, and the nuclei are intensively shrunk and hyperchromatic; (3) histiocytes may be observed; and (4) the cyst-lining cells have an abundant, spindle blue pale cytoplasm and enlarged nuclei. These nuclei are round or elongated with anisokaryosis. Their chromatin is pale; they show prominent nucleoli and an underlined nuclear membrane (Fig. 2).



Colloid Goiter, Cytological Findings, Fig. 3 Large drops of blue dense colloid without any other component on this slide; considering the quantity of colloid, this nodule can be classified as benign (colloid nodule) (LBC Hologic®, Papanicolaou staining ×40)

Immunophenotype

The follicular cells are positive for thyroglobulin and TTF1. They are negative or weakly positive for CK19, Galectin-3, and HBME1. Usually immunocytochemistry is not useful.

Molecular Features

BRAF, RAS, RET/PTC, and PAX8/PPAR-γ mutations are not found in benign colloid nodules. Usually it is not necessary to apply these tests on FNA since the cytological diagnosis is relevant. It is useful in cases of "indeterminate cytology."

Differential Diagnosis

A diagnosis of colloid nodule is usually quite easy. Following the BSRTC, it is nevertheless essential to notice that a minimum of six sheets of well-preserved follicular cells are required to fall from the nondiagnostic category to the benign category. When some few microfollicles are observed and maybe some discrete nuclear changes such as anisokaryosis without other abnormalities, then a diagnosis of follicular lesion of undetermined significance may be proposed. Finally there is a risk of a pitfall with parathyroid adenoma included in the thyroid gland since parathyroid cells and follicular cells do not appear obviously different.

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Congenital Mesoblastic Nephroma, Cytological Findings

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Synonyms

Congenital mesoblastic nephroma; Fetal mesenchymal hamartoma of kidney; Infantile renal fibrosarcoma; Leiomyomatous hamartoma of the kidney

Definition

Congenital mesoblastic nephroma (CMN) is a low-grade fibroblastic sarcoma – low-risk tumor European Society of Paediatric Oncology (SIOPE-2001-Revised working classification of renal tumors of childhood).

It represents 3–10% of all pediatric tumors and is the most common congenital renal neoplasm. Initially classified as a hamartoma and misdiagnosed as Wilms tumor, it is now believed, since Bolande et al. in 1967, that it represents a different entity that originates from the renal mesenchyme (Bolande et al. 1967).

Clinical Features

- **Incidence**
  Detection before birth is possible with fetal scan. During pregnancy it can be the cause of nonimmune fetal hydrops (due to excessive fetal urine production) and hydramnios with consequent premature delivery. The most typical presentation is that of a palpable abdominal mass. In some patients hematuria, hypertension, and hypercalcemia have been described.
  Radiography is poorly sensitive and postnatal; ultrasonography is usually the first study performed whenever there is an abdominal palpable mass. CT scans demonstrate a solid mass arising from the kidney and centered in the renal sinus. Cystic, necrotic, and hemorrhagic areas may be seen, mainly in cellular variant.
- **Age**
  CMN is typically diagnosed in the first 3 months of life, and 90% of the cases are diagnosed in the first year.
- **Sex**
  Some studies show a male predominance.
- **Site**
  CMN is a unicentrically primary tumor of the kidney, centered near the renal hilus, and there are no reports of cases outside the kidney.
- **Treatment**
  Excision is the elected therapy.

- **Outcome**
  Locally, the tumor can show invasion, but metastases to distant organs such as the brain, bone, and lungs are uncommon.
  Most cases of mesoblastic nephroma have an excellent prognosis with a rate of only 5% of relapses, most due to incomplete excisions.

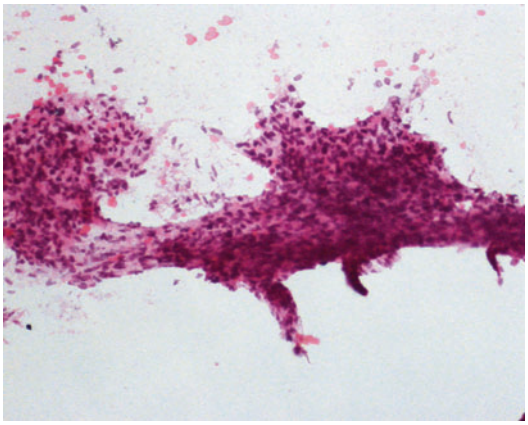
Macroscopy

Mesoblastic nephromas are centered near the hilus involving the renal sinus. Generally these tumors are macroscopically well circumscribed, with a whitish color and with a whorled texture. Not uncommonly they can be cystic, with hemorrhagic areas and necrosis.

Two different morphologic and molecular types are described: classical and cellular type. In the classical type (24% of the cases), borders are more infiltrative, and the tumor can extend into the adjacent kidney or even to the perirenal soft tissue. Attention must be paid to macroscopic evaluation of the hilar region in order to evaluate invasion of hilar fat. In the cellular type (40–60%), borders are more pushing, and hemorrhages as well as necrosis are more common.

Microscopy

In the classical type, fascicles and whorls of bland fibroblastic and myofibroblastic-like cells simulate an infantile fibromatosis (Fig. 1). Mitosis and necrosis are rare. In some cases extramedullary hematopoiesis can be seen. In the periphery the tumor entraps normal renal tubules that can acquire primitive appearance (embryonal metaplasia). These tubular cells with reactive changes can be observed sometimes deeply entrapped in the tumor and can have atypical aspects leading to misdiagnosis. Entrapped tubules are **E**pithelial **M**embrane **A**ntigen (EMA) and **C**luster **D**ifferentiation CD57 positive and CD56 **N**eural **C**ell **A**dhesion **M**olecule (NCAM) negative. These antibodies are helpful in distinguishing mature from immature tubular epithelium.



Congenital Mesoblastic Nephroma, Cytological Findings, Fig. 1 Mesoblastic nephroma – classic type. A fragment of stromal tissue with spindle cells lying in a cohesive fragment. Nuclei are spindle, chromatin is homogeneously dispersed, and nucleoli are inconspicuous. Cytoplasm is scarce and poorly defined (H&E, 400x)

Cellular type is very similar to an infantile fibrosarcoma. The borders are pushing and the tumor is composed of dense and cellular sheets of bland, plump cells with large vesicular nuclei and fine chromatin. Nucleoli can be present and prominent. Mitoses are variable and can be numerous. Necrosis can be impressive. Mixed histological patterns can also be seen.

Cytological smears reproduce histological features and show a variable cellularity, with naked nuclei and spindle cells singly or in cohesive fragments. Nuclei are oval in the cellular type or more spindle in the classical type. Chromatin is homogeneously dispersed with inconspicuous nucleoli. Cytoplasm is scarce and poorly defined. The background is generally clear although the presence of mucous-like material or necrosis has been described (Geisinger et al., 2004).

Immunophenotype

These tumors are immunoreactive for vimentin and smooth muscle actin (classic type).

Wilms tumor-WT1 antibody can show a cytoplasmic positivity, and desmin, CD34, CD10, and CD56 (NCAM) are negative.

Molecular Features

Polysomies of chromosome 11, 17, and 20 are seen only in the cellular variant. The cellular variant is also characterized by a constant t(12; 15) (p13;q25) with consequent fusion of ETV6/NTRK3 gene (Argani and Ladanyi, 2003).

This translocation is shared with infantile fibrosarcoma and secretory breast cancer. In this last tumor there is no evidence of trisomy 11 which is found in virtually all cases of congenital fibrosarcoma and ▶ congenital mesoblastic nephroma (Knezevich et al. 1998).

Differential Diagnosis

Cytological pitfalls in the diagnosis are posed with Wilms tumor (stromal predominant), ▶ clear cell sarcoma, metanephric stromal tumor, and ▶ rhabdoid tumor of the kidney.

Pure stromal cases of Wilms tumor are rare but may be a problem, mainly in specimens after chemotherapy. Chemotherapy often ablates immature and proliferating component as blastema and spares mature stromal areas. Multiple samples can solve this problem as pure undifferentiated stromal tumors are rare. In Wilms tumor mesenchyme is looser, sometimes myxoid, and in CMN stromal fragments are more cellular and disorganized.

One must also be aware of embryonal metaplastic lesions entrapped (CD57 positive and CD56 NCAM negative) in the periphery of CMN and not misinterpret as immature tubules (CD56 NCAM positive) of Wilms tumor.

▶ Clear cell sarcoma of the kidney has a characteristic metachromatic mucoid-like material rich in glycoprotein's seen in Giemsa-stained slides; the nuclei have a more vesicular chromatin with nuclear grooves. In typical smears of CCS, the presence of arborizing capillaries or fragments of magenta stroma can help characterize this tumor. Immunostains are not helpful distinguishing these entities.

Metanephric stromal tumor (MST) is diagnosed in children older than 3 years and shows typical concentric onionskin rings around entrapped vessels and renal tubules (aspect only appreciated in histology). CD34 is positive in MST.

Some CMN (plump cell type) can have polygonal cells with rhabdoid features raising the differential diagnosis with ▶ rhabdoid tumor of the kidney. If extensive sampling or age is not enough to distinguish these two entities, then one can always evaluate the loss of **I**ntegrase **I**nteractor 1 (INI1) protein expression by immunohistochemistry, which is highly specific of rhabdoid tumor.

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Conjunctiva Cytology, General Aspects

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Description

Cytological samples of conjunctiva may be obtained by scraping, brushing, or impression

cytology. Conjunctival scraping requires local anesthesia and should be performed by trained ophthalmologists. Scraping may be performed using a small platinum spatula (Swedish Dissector) or a scalpel blade. Conjunctival brush biopsy may also be carried out under local anesthesia using a small cytobrush. Impression cytology of the conjunctiva can be performed by placing cellulose acetate strips on the surface of the conjunctiva or the cornea and pressing gently. The strip is then removed, fixed in absolute ethanol or glacial acetic acid, and stained using PAS/Papanicolaou stain; biopore membranes have also been used.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Conjunctival Inflammatory Lesions, Cytological Findings

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Description

Allergic and vernal conjunctivitis are characterized by a prevalence of eosinophils in the inflammatory infiltrate. Bacterial and viral infections are generally diagnosed on the basis of clinical presentation or sometimes by microbiology. Nonetheless, cytological features may be helpful for a presumptive diagnosis, mainly in clinically unexpected cases and in the general management of the patients. Infections by verruca vulgaris, measles, and herpes may be suggested by specific cytological features. Trachoma is a "historical" bacterial conjunctival infection caused by Chlamydia trachomatis. The cytological features are almost the same as those described in the female genital tract and are characterized by cytoplasmic basophilic inclusions with halos. Cytological features of Candida, Aspergillus, and mucormycosis conjunctivitis have also been described; diagnosis and treatment must be timely and effective because of the risk of corneal damage.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Conjunctival Lymphoma, Cytological Findings

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Description

Lymphomas may arise from conjunctival-associated lymphoid tissue (CALT); cytopathological features are similar to those of other mucosa-associated lymphomas (MALT).

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
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- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
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- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
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- ▶ Meningioma, Cytological Findings
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- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Conjunctival Melanocytic Tumors, Cytological Findings

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Definition

Spectrum of melanocytic proliferation ranging from benign to malignant tumors.

Clinical Features

- **Incidence**
  The most common tumors of the conjunctiva.
- **Age**
  Not applicable.
- **Sex**
  No gender predilection.
- **Site**
  Conjunctiva.
- **Treatment**
  Nevi: follow-up. Primary acquired melanosis and malignant melanoma: surgery.
- **Outcome**
  Malignant melanoma: mortality at 10 years – 15–40%.

Macroscopy

Melanocytic nevi of the conjunctiva are classified in almost the same way as those of the skin, i.e., as junctional, subepithelial, and compound. Conjunctival nevi may increase in size, which may occur in puberty, or change shape, as in the case of "irritated" nevi or in malignant transformation.

Primary acquired melanosis (PAM) may be considered as the conjunctival counterpart of cutaneous Hutchinson lentigo.

Malignant melanomas may arise ex novo or through transformation of preexisting nevi or acquired melanosis.

Microscopy

Microscopically melanocytic nevi are formed by pigmented polygonal cells and are more cellular than their epidermal counterparts.

The histological features of PAM are quite variable even within the same lesion and range from small monomorphous melanocytes which line the basal layer to atypical nucleolated epithelioid cells. Pagetoid infiltration of the epithelium is indicative of malignant transformation in most cases.

FNA of a conjunctival malignant melanoma has been described; smears show dispersed tumor cells with eccentric nuclei, coarsely granular chromatin, and prominent nucleoli; occasional or prominent cytoplasmic melanic granules and spindle-shaped cells may be present. Impression cytology and Biopore membrane have yielded excellent diagnostic results, predicting the histological diagnosis by detection of superficial atypical melanocytes and their proportion relative to the conjunctival cells.

Differential Diagnosis

Melanocytic nevi, PAM, ▶ malignant melanoma

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Conjunctival Papilloma, Cytological Findings

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Description

Benign epithelial tumors are principally represented by papillomas, which are composed of a fibrovascular stalk covered by acanthotic squamous epithelium; cytological diagnosis is rarely requested.

Cross-References

▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
▶ Conjunctiva Cytology, General Aspects
▶ Conjunctival Inflammatory Lesions, Cytological Findings
▶ Conjunctival Lymphoma, Cytological Findings
▶ Conjunctival Melanocytic Tumors, Cytological Findings
▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
▶ Cornea Cytology
▶ Cytology of the Orbit and Ocular Adnexa
▶ Eyelids Cytology, General Aspects
▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Meningioma, Cytological Findings
▶ Orbit Cytology, General Aspects
▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
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Conjunctival Squamous Cell Carcinoma, Cytological Findings

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Synonyms

SCC

Definition

Malignant conjunctival tumors are represented by ► Squamous Cell Carcinoma and its precursors; the term ocular surface squamous neoplasia (OSSN) is generally used to describe a spectrum of lesions, ranging from mild dysplasia to carcinoma in situ, which may arise on the conjunctiva and cornea.

Clinical Features

- **Incidence**
  Most common conjunctival malignancy, yearly incidence – 1-3 per 100,000.
- **Age**
  Incidence associated with middle old age.
- **Sex**
  No gender predilection.
- **Site**
  Conjunctiva.
- **Treatment**
  Surgery, cryotherapy, and chemotherapy.
- **Outcome**
  Rarely metastasizes; it can invade into the eye.

Macroscopy

Leukoplakia or Papilloma like or Pink nodule on the eye surface.

Microscopy

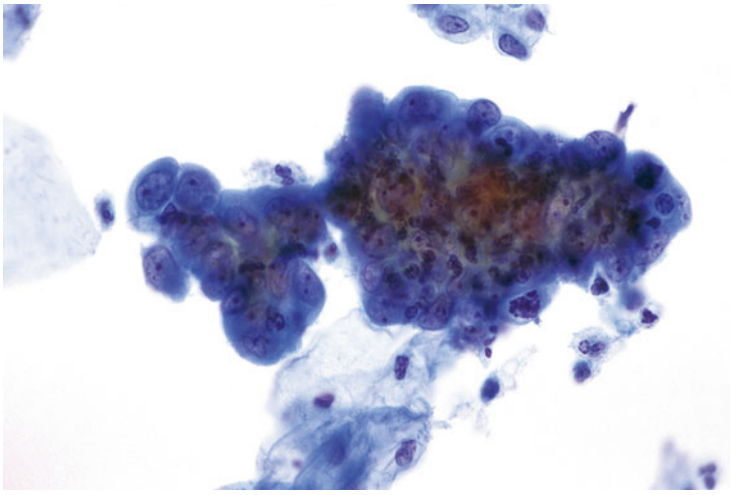
Smears show immature cells with increased nucleus/cytoplasm ratio; nuclei are enlarged and hyperchromatic with irregular contours, coarse chromatin, and prominent nucleoli (Fig. 1). Many high-grade lesions and some invasive carcinomas may show superficial keratinization, which makes the cytological diagnosis more difficult.

Immunophenotype

Not applicable

Molecular Features

Not applicable



Conjunctival Squamous Cell Carcinoma, Cytological Findings, Fig. 1 Conjunctival scraping of an intraepithelial neoplasia: groups of malignant epithelial cells with overlapping and nuclear atypia. Benign squamous cells are present on the edges (Diff-Quik stain 270×)

Differential Diagnosis

The differential diagnosis must include conjunctival squamous metaplasia (abundant cytoplasm, reduced nuclear/cytoplasmic ratio, pycnotic nuclei) and parakeratosis (dyskeratotic cells and keratohyaline granules). A quite high correlation has been found between cytology diagnoses and corresponding histological controls, while the greatest probability of false-negatives is found in keratinizing squamous cell carcinoma

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Gland Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
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- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Cornea Cytology

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Description

Corneal cells strongly resemble the intermediate squamous cell of the female genital tract. Inflammations are generally diagnosed and treated on the basis of clinical presentation, and cytology has been used to diagnose unusual clinical presentations or rare infections such as actinomycosis, blastomycosis, or acanthamoebic keratitis, especially when the organisms are superficial. Corneal neoplasia is represented by OSSN as reported in the conjunctiva.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
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- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings

- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Curschmann's Spirals

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Definition

Coiled, basophilic mucinous fibrils sometimes found in the sputum and tracheal/bronchial washings.

Clinical Features

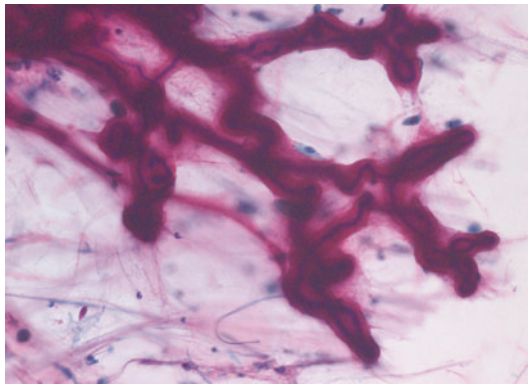
- **Incidence**
  Curschmann's spirals are frequently found in the secretions of the respiratory tract in patients with a high viscosity of the secretion. Rarely, Curschmann's spirals may be encountered in cervicovaginal secretion.
- **Age**
  Any age group may be affected.
- **Sex**
  There is no specific male or female predominance.
- **Site**
  Curschmann's spirals are usually found in the secretions of the respiratory system. However, they have been described in vaginal secretions as well.
- **Treatment**
  Curschmann's spirals indicate an increased viscosity of secretions. They themselves do not need to be treated. However, the underlying disease may require treatment.
- **Outcome**
  The outcome depends on the underlying disease.

Macroscopy

Viscous mucus can be present. However, macroscopically, the presence or absence of Curschmann's spirals cannot be noticed.

Microscopy

Curschmann's spirals can be composed of twisted, spirally arranged mucin with loose or tight twists, or they can form tightly coiled fibrils (see Fig. 1). They are most often basophilic, with a width of 25 μm and a length of several hundred μm. Curschmann, who first described the spirals in 1882, suggested that they are formed in the finer bronchioles. Probably they develop due to an imbalance of



Curschmann's Spirals, Fig. 1 A Curschmann's spiral shows a twisted, basophilic structure (Papanicolaou, 60×)

sulfated and non-sulfated sialomucins in the mucus, which leads to an increase of the viscosity. Curschmann's spirals are mostly found in patients suffering from asthma and chronic bronchitis, often in association with Charcot-Leyden crystals. Curschmann's spirals have been described in cervicovaginal secretion as well; there the origin is unknown.

Differential Diagnosis

Foreign bodies may be mistaken for Curschmann's spirals.

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Cyst of Salivary Gland, Cytological Findings

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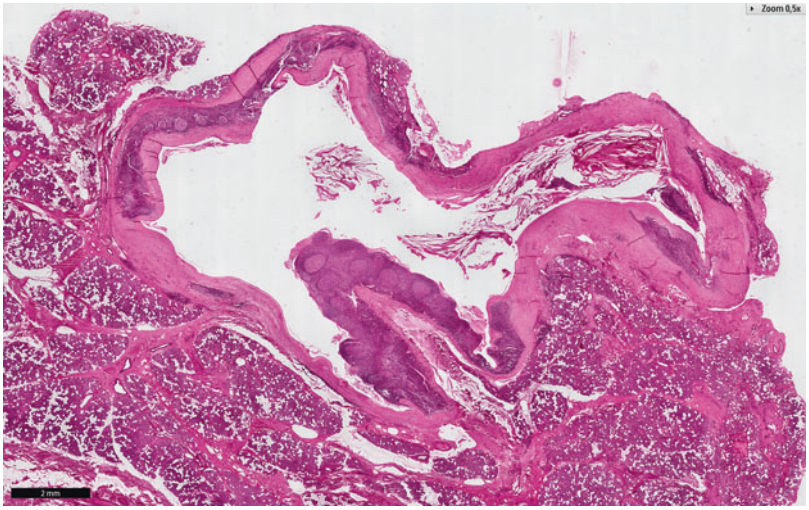
Definition

Cystic dilatations in salivary glands have multiple etiologies (Barnes et al. 2005; Ellis and Auclair 2008). Entities showing cystic spaces are the following: salivary duct cyst (DC), HIV-associated salivary gland disease (HSC), lymphoepithelial cyst (LEC) (Fig. 1), polycystic disease of the parotid gland (PD), mucocele (MC), and mucus retention cyst (MRC). Etiology is also various and related to inflammation, retention, and malformation or may simply remain unknown.

Clinical Features

- **Incidence**
  Cystic dilatations are rare. HSC are usually bilateral lymphoid hyperplasia with lymphoepithelial cysts and lesions and present in less than 10% of HIV-affected patients (Michelow et al. 2012).
- **Age**
  Cysts depending of etiology may be in different ages. DC usually occurs in patients older than 30 years, HSC in young adults, and LEC in fourth to seventh decades. PD usually occurs in children or young adults whereas MC mainly affects patients younger than 30 years. MRC's peak of incidence is in seventh and eighth decades.
- **Sex**
  There is no sex predilection of DC, HSC, MC, and MRC. There are a male predilection in LEC and a female predilection for PD.



Cyst of Salivary Gland, Cytological Findings, Fig. 1 Lymphoepithelial cyst of the parotid gland in HIV-positive patient. Note the presence of numerous crystalloids (H&E stain)

- **Site**
  DC, HSC, LEC, PD, and MRC usually occur in the parotid gland and MC in the lower lip and may occasionally be intraoral.
- **Treatment**
  Aspiration is frequently a sufficient therapeutical modality. In recurrent cases the treatment is surgical.
- **Outcome**
  Recurrences are rare.

Macroscopy

Cysts are unilateral or bilateral, unique or multifocal. Intracystic content varies from viscous, mucous, to watery.

Microscopy

Cytology smears in salivary cysts (SC) are usually fluid and hypocellular.

Aspiration of a SC may lead to disappearance of the lesion. Smears are composed of a watery or mucoid fluid with occasional inflammatory and necrotic material. Oncocytic cells, squamous cells, ciliated cells, crystalloids, and macrophages may be present in various proportions (Fig. 2).

Immunophenotype

There is no utility to use immunohistochemistry.

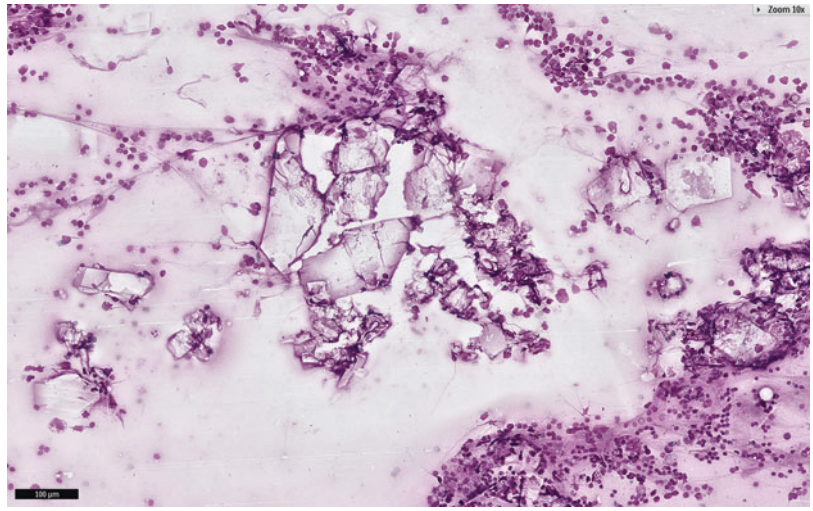
Molecular Features

There is no utility to use molecular studies except to make the differential diagnosis between mammary analogues of secretory carcinoma (MASC), harboring a t(12;15) (p13;q25) translocation which leads to *ETV6-NTRK3* fusion detectable by FISH, which is absent in the cystic forms of adenoma or carcinoma (Weinreb 2013).

Differential Diagnosis

SC should be differentiated from axial congenital lesions such as thyroglossal cysts, axial thymic cysts, dermoid cysts, and branchial cleft cysts (when located laterally). The main differential diagnosis concerns low-grade mucoepidermoid carcinoma (MEC), Warthin's tumor (WT), and cystic metastasis from squamous cell carcinoma (SCC).

Like SC, low-grade MEC may yield cystic mucoid fluid. Squamoid cells and intermediate



Cyst of Salivary Gland, Cytological Findings, Fig. 2 Lymphoepithelial cyst of the parotid gland. Numerous cell debris and crystalloids (MGG stain)

cells allow making of the correct diagnosis. Mucus-secreting cells may be misdiagnosed as macrophages. Inversely, crystalloids and stones are extremely rare in MEC (Klijanienko and Vielh 1997a). WT is also composed from a cystic fluid with dense and mucinous background. Typical WT contains oncocytic clusters with mast cells which readily differentiate from SC (Klijanienko and Vielh 1997b). Squamous cell carcinoma usually contains numerous squamous malignant cells and keratin debris (Klijanienko and Vielh 1998).

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Cytology of the Orbit and Ocular Adnexa

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Description

The orbit and ocular adnexa, which cytopathologists are rarely requested to investigate, are one of the most complex and difficult anatomical regions to investigate cytologically. Furthermore, the scarcity of routine orbit and ocular adnexa cytology increases the difficulties of cytopathologists who often have to deal with

scanty cellular samples from different and sometimes complex or rare pathologies; moreover, the specific clinical and anatomical features require close cooperation between ophthalmologists and cytopathologists. In fact, whereas cytopathologists routinely perform fine needle cytology (FNC) on much of the palpable and impalpable lesions by themselves, in this specific field the insertion of the needle is an almost exclusive task of ophthalmologists. The cytopathologist's role is that of making smears, evaluating adequacy, managing diagnostic material, selecting ancillary techniques, and making the final diagnosis. Finally, orbital and ocular adnexa cytology, as in other regions, has been improved by immunocytochemistry (ICC), flow cytometry (FC) fluorescence in situ hybridization (FISH), and molecular techniques; however, the usage of these procedures is often impeded by the scanty material obtainable, the management of which further increases the complexity of the cytological approach.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Cytomegalovirus, Cytological Findings

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Synonyms

CMV; Cytomegalovirus

Definition

Cytomegalovirus (CMV) (from the Greek *cyto-*, "cell," and *-megalo-*, "large") is a herpes-type virus of the Herpesviridae family and is a component of a family of eight human herpes viruses, assigned as HHV-5 (human herpes virus 5) (http://www.theviraldiseases.com/cytomegalovirus-infection-cmv-facts.html).

Clinical Features

In healthy individuals, CMV can be manifested with mild or absence of symptoms whereas it can cause serious illness in the immunosuppressed or in the fetus/newborn. There are many possibilities of transmission essentially summarized by a sexual contact, inborn, via blood items or transplants and people-to-people spread.

Microscopy: Cervical Cytology

Endocervical cells are susceptible to CMV infection (Byard et al. 1991), but the cytological features caused by the CMV are rarely seen in cervical cytology preparations.

While an infected cell in the early stages will show small inclusion bodies, in the later stages of the infection, cells have an enlarged nucleus containing a large eosinophilic inclusion which is surrounded by narrow perinuclear halo which is often referred to as having an "owl's eye" appearance (Mattes et al. 2000).

CMV and Cervical Cancer

CMV is thought not to be involved directly in the oncogenic processes of cervical cancer, but it might enhance the possibility of oncogenesis or infect cancer tissues opportunistically (Yang et al. 2004; Al-Daraji and Smith 2009).

Detection of CMV

The detection of CMV by cervical cytology is very unreliable. Reporting of cells showing features of CMV can be of importance especially in pregnant women as CMV infection can be harmful to the health of the fetus.

CMV is generally detected by testing the patient for CMV IgM and IgG antibodies. Although the determination of the specific virus is made by viral culture, the morphological detection of CMV RNA can be demonstrated using in situ hybridization, immunohistochemistry, or a combination of the both methods.

Both qualitative and quantitative polymerase chain reaction (PCR) testing for CMV are available, allowing clinicians to monitor the viral load of CMV-infected patients.

CMV pp65 antigenemia test is an immunofluorescence-based assay which utilizes an indirect immunofluorescence technique for identifying the pp65 protein of CMV in peripheral blood leukocytes.

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Cytospin Technique

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Definition

Cytospin technique is a method of performing thin-layer preparation from fluids through a cytocentrifugation process.

Principle

During the procedure, the material is centrifuged and the cells are deposited onto a vertical microscope slide. This procedure permits a cell separation from the fluid medium.

Methodology

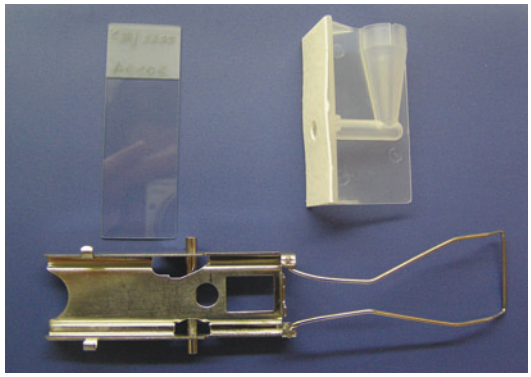
Samples are centrifuged at 600–1,500 rpm, in special plastic sample chambers (Fig. 1) composed by a filter card, a glass slide and a filling port

Cytospin Technique, Fig. 1 Sample Chamber

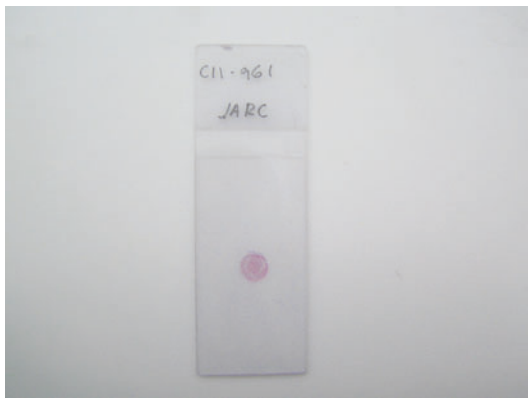
(Funnel). These chambers are held in position in the centrifuge device with special stainless steel cytoclips. During the procedure the cells that are denser then the suspending fluid, are projected towards the glass slide crossing the gap formed by the filter laid between the funnel and the glass slide (Fig. 2). The suspended fluid is absorbed by the filter and the cells are sedimented within a 32 mm (Fetsch et al. 2002) area of the glass slide (Fig. 2) with an unquestionable advantage of reducing screening time. This is the most common method used to perform cytospins and it is commercialized by Shandon (Fig. 3) (Finger et al. 2006).

Quality Aspects

Cytospins can be air-dried, fixed in alcohol or freeze and can be stained with any histochemical or immunocytochemical method (Fetsch et al. 2002). Cytospins are also suitable for molecular methods as Fluorescence in situ hybridization



Cytospin Technique, Fig. 2 Shandon equipment for cytospins preparation. These cyto-funnels **laid** a thin layer of cells onto a microscope slide in a well defined **6 mm area** (see Fig. 3). The Stainless steel funnel clips hold the cytology funnels against the slide during the centrifugation process



Cytospin Technique, Fig. 3 The final monolayer slide with the material consistent with a clearly defined 6 mm area

(FISH) or chromogenic in situ Hybridization (CISH) technique (Pusztaszeri et al. 2011).

The absorption of the suspension fluid of the sample is particularly useful in immunostains of lymphoproliferative disorders.

Applications

In many institutions fine needle aspiration cytology (FNAC) is used as a first line approach to evaluate lymphadenopathies and is associated to high rate of conclusive cytological diagnoses in the assessment of Hodgkin disease or large cell non Hodgkin lymphomas (79–90%).

Immunocytochemical is an essential ancillary technique determining clonality and antigenic characteristics of specific subtypes of lymphomas. Detection of clonality represents the essential step in the differential diagnosis of reactive lymphadenophaties and lymphoma. Lymphomas develop from a single clone of B or T cells, so that, all neoplastic lymphocyte population present an identical immunoglobulin (B cells) or *TCR* gene rearrangement (T cells).

In B cell lymphomas clonality can be demonstrated indirectly through immunocytochemistry, establishing the presence of a light chain restriction. Generally cytological smears obtained from fine needle aspiration of lymph nodes, display a disturbing background composed of immunoglobulin's and cytoplasmic debris that stain intensely with lymphocytic directed immunostains (CD45, CD20, CD3, etc. . . .), making it difficult to evaluate. Cytospins preparations lack this confounding background, improving immunocytochemical results with these antibodies (Barroca et al. 2008). The application of Cytospins in immunocytochemistry is not confined to lymphoma diagnosis. In liquid cytology and whenever the sample is not sufficient to perform cytoblocs, cytospins may be held for further ancillary techniques usefull in diagnostic, prognostic studies or even in the therapeutic stratification (see examples). In cytospin, the cell concentration by centrifugation facilitates the accomplishment of molecular techniques such as FISH and CISH.

Examples

See Table 1-Examples of the application of ancillary techniques in the diagnosis, prognosis and therapeutics of solid tumours, validated for cytospins.

Cytospin Technique, Table 1 Examples of the application of ancillary techniques in the diagnosis, prognosis and therapeutics of solid tumours, validated for cytospins

	Cytologic material	Ancillary techniques; clinical significance and important markers
Lung (Warth et al. 2013); (Rho et al. 2013)	EBUS, FNB, washes, serous fluids	Immunostains- diagnosis: SCC/NSCC; ADC/SqCC
		FISH-prognosis and therapeutics: EGFR; ALK
Breast	FNB	Immunostains- Treatment and prognosis Estrogen,progesterone Receptor,HER 2
		CISH: Treatment and prognosis (HER 2)
RMS (Das et al. 2006)	FNB	Immunostains- diagnosis
		FISH- diagnosis and prognosis: t(2;13)(q35;q14); t(1;13)(p36;q14)
SS[a]	FNB	Immunostains- diagnosis
		FISH-diagnosis and prognosis: t(X;18)(p11;q11); t(X;18)(p11;q11); t(X;18)(p11;q13); t(X;20)(p11;q13)
EWING[a] (Hattinger et al. 2000)	FNB	Immunostains- diagnosis
		FISH-diagnosis: t(11;22)(q24;q12)
Liposarcoma	FNB	FISH- diagnosis: Mdm2
Myxoid liposarcoma	FNB	FISH-diagnosis: t(12;22)(q13;q12)
DSRCT	FNB	FISH-diagnosis: t(11;22)(p13;q12)
Extraskeletal myxoid chondrosarcoma (Jakowski et al. 2007)	FNB	Immunostains-diagnosis;
		FISH- Prognosis: t(9;22)(q22;q12); t(9;17)(q22;q11); t(9;15)(q22;q21); t(9;22)(q22;q15)
NB	FNB	FISH-Prognosis and therapeutics: N-Myc; del1p; 17q gains
Renal cell carcinoma	FNB	FISH-diagnosis: t(X;17)(p11;q25)
ASPS	FNB	FISH-diagnosis: t(X;17)(p11;q25)
Congenital fibrosarcoma	FNB	FISH-diagnosis: t(12;15)(p13;q25)
NUT carcinomas	FNB	FISH- diagnosis; t(15;19)

RMS rhabdomyosarcoma, *SS* synovial sarcoma, *FNB* fine needle biopsy, *EBUS* endobronchial ultrasound biopsy, *ADC* adenocarcinoma, *SqCC* squamous cell carcinoma, *SCC* small cell carcinoma, *NSCC* non small cell carcinoma, *DSRCT* desmoplastic small round cell tumour, *NB* neuroblastoma, *RCC* renal cell carcinoma, *ASPS* Alveolar soft part sarcoma
[a]Kumagai et al. (2010)

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D

Ductal Breast Carcinoma, Cytological Findings

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Synonyms

Invasive carcinoma of no special type (NST)

Definition

The invasive breast carcinoma of NST, known as ductal carcinoma, is a heterogeneous group of tumors that fail to exhibit characteristics to achieve classification as a specific histological type. The term "ductal" is not related to the coming from the duct, since most carcinomas arise in the terminal duct-lobular unit but it represents a growth pattern associated with the cell types.

Clinical Features

- **Incidence**
  It represents the most frequent invasive carcinoma of the breast, comprising between 41% and 77% of all breast carcinomas. This range reflects the difficulties and non-univocal classification among pathologists in the presence of cancers with component showing special patterns.
- **Age**
  The median age is around 50–69 y/o with about 6% in women younger than 39 y/o.
- **Sex**
  It is more than 200 times more common in women than men. In the latter group the patients are usually old.
- **Site**
  The lesion may be found anywhere in the breast, but the outer upper quadrant is the most frequent site.
- **Treatment**
  The care of patients with breast cancer involves several disciplines including surgery, in general limited surgery with conservation of the breast, sentinel node biopsy, limited radiation therapy, and appreciate adjuvant therapy with tailored targeted therapies.

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4

- **Outcome**
  The 5-year survival is approximately 55–65%. An adverse outcome seems to be associated with triple-negative phenotype.

Macroscopy

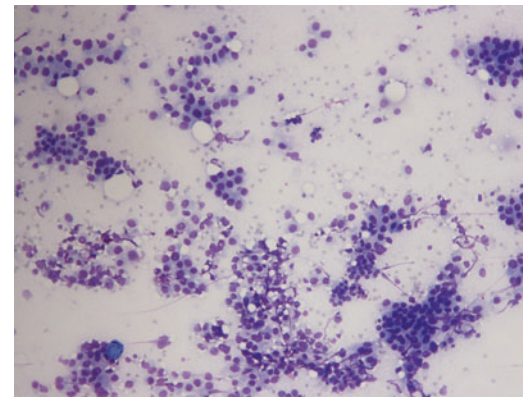
These tumors have no specific gross features. The tumor may vary in size from one to several centimeters and presenting as a firm, discoid, hard mass with irregular edges.

Microscopy

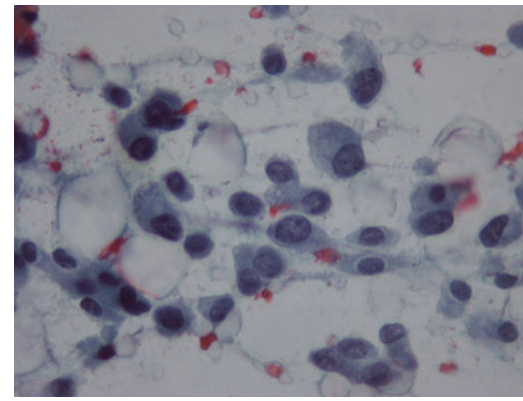
The aspirates of infiltrating duct carcinoma show some key features: (1) abundant cellularity represented by (2) loosely cohesive or individual malignant cells, (3) the cells are organized in three-dimensional clusters or syncytial pattern without any type of cellular polarity and with nuclear molding in the (4) absence of naked bipolar nuclei, and (5) tumor diathesis is present. The cells are usually large in size with anisonucleosis, irregular nuclear borders, and high N/C with granular chromatin and small or prominent nucleoli. The cytoplasm is basophilic and granular. The cells of duct carcinoma are usually bigger and more hyperchromatic than the lobular variant. The histological feature shows cell growing in sheet, nest, cords, glands, or trabeculae with infiltrative behavior through the surrounding fibrotissue. The high-grade lesions show usually a central core of fibrosis. Necrosis is present in 33% of tumors as well calcification which can be identified in around 60% of cases. Many cases show elastosis (Figs. 1–4).

Immunophenotype

The neoplastic cells are positive for CK7, CAM 5.2, and EMA. A high percentage of cases are also positive for "luminal" CK 8/18 and CK 19. Details about the predictive markers ER, PR, HER2, and Ki-67 are in the histopathology session.



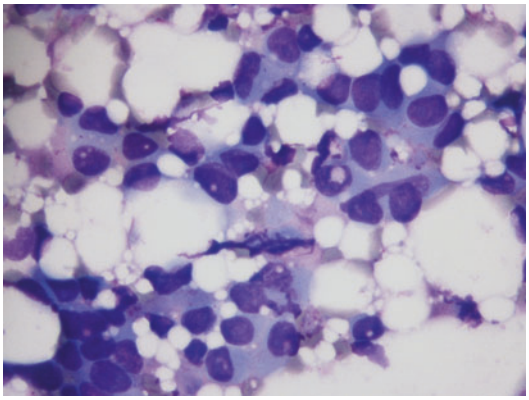
Ductal Breast Carcinoma, Cytological Findings, Fig. 1 Invasive ductal carcinoma. Note a high cellular smear with loosely cohesive malignant cells (Giemsa stain)



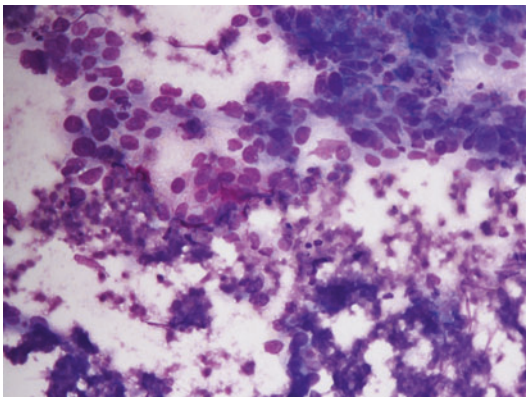
Ductal Breast Carcinoma, Cytological Findings, Fig. 2 Invasive ductal carcinoma. Note isolated malignant cells with preserved cytoplasm (Papanicolaou stain)

Molecular Features

Details about the molecular classification of breast carcinomas are provided in other sessions of the encyclopedia. Approximately 60% of invasive carcinomas are strongly positive for ER. In general grade 1 carcinomas are positive for ER and PR, whereas high grade may be negative. HER2 overexpression plays an essential prognostic role as it emerged from several studies underlying that 97% of cases with unequivocal HER2 overexpression are invasive duct carcinoma of grades 2 and 3. Cytological



Ductal Breast Carcinoma, Cytological Findings, Fig. 3 Invasive ductal carcinoma. Atypical cells characterize by anisonucleosis, irregular nuclei borders, and high N/C ratio (Giemsa stain)



Ductal Breast Carcinoma, Cytological Findings, Fig. 4 Invasive ductal carcinoma. Note smear with tumor diathesis in the background

features of high nuclear grade, necrosis, and presence of inflammatory cells are associated with triple-negative phenotype.

Differential Diagnosis

Well-differentiated carcinomas should be differentiated from benign lesions. Absence of myoepithelial cells among the epithelial group and absence of naked nuclei in the background of the smears favor malignancy in these cases. In some cases, fibroadenomas may show large irregular nuclei with nucleoli; the presence of the two cell populations of epithelial and myoepithelial cells is the major criterion of identification. In situ duct carcinoma can also display myoepithelial cells even if in a less numerous percentage.

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Ductal Carcinoma of Salivary Gland, Cytological Findings

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Synonyms

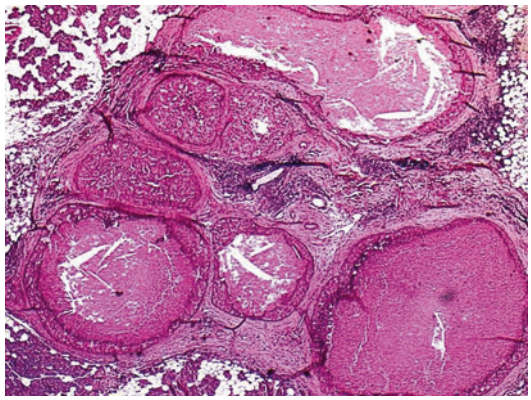
Salivary duct carcinoma

Definition

Ductal carcinoma of salivary gland (SDC) is a high-grade, aggressive salivary adenocarcinoma (Barnes et al. 2005; Ellis and Auclair 2008) morphologically resembling breast in situ and infiltrating ductal cell carcinoma (Fig. 1). Many cases are metastatic at initial presentation.

Clinical Features

- **Incidence**
  SDC represents less than 1% of salivary gland tumors.
- **Age**
  Adult's age. Most of the tumors arise after 50 years.
- **Sex**
  There is a male to female predominance (2.5 to 1).
- **Site**
  The parotid gland is the site of occurrence in more than 85% of cases.
- **Treatment**
  Surgery and chemotherapy followed by chemotherapy are usually used.
- **Outcome**
  SDC is a high-grade malignancy with elevated rate of local recurrences and metastases.



Ductal Carcinoma of Salivary Gland, Cytological Findings, Fig. 1 Salivary duct carcinoma. High-grade adenocarcinoma morphologically resembling breast in situ and infiltrating ductal cell carcinoma (H&E stain)

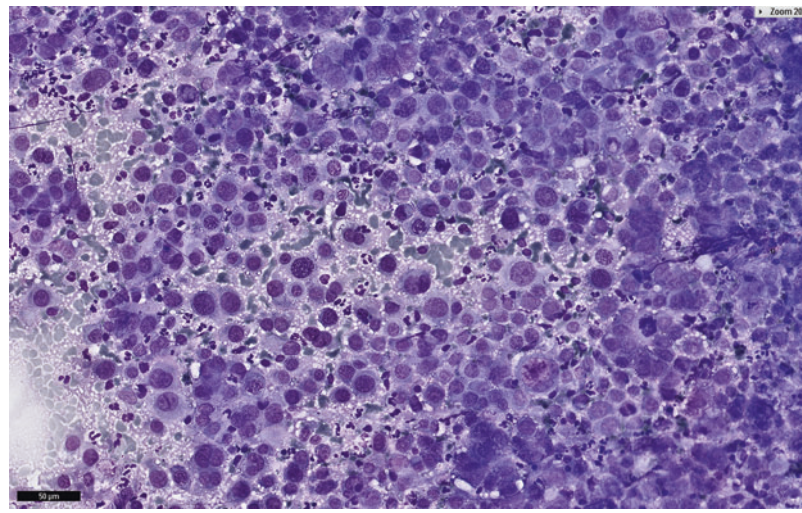
Macroscopy

Usually, SDCs are multinodular with poorly limited grayish-white masses. Necrotic and hemorrhagic areas are common.

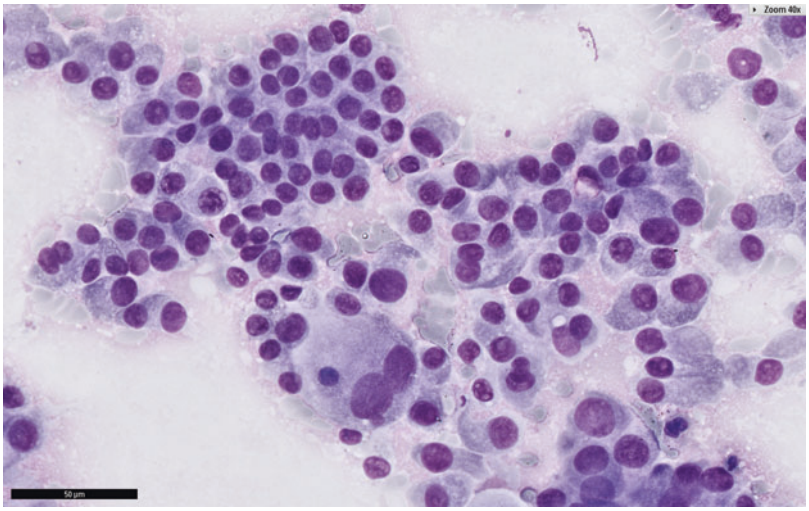
Microscopy

Cytology smears (Klijanienko and Vielh 1998a) in salivary duct carcinomas (SDC) are usually hypercellular and cell-rich and stroma-poor. SDC belongs to the group of tumors exhibiting predominant epithelial cell morphology.

Cytologically, SDCs are similar to high-grade ductal adenocarcinoma of the breast. Cells are either isolated or grouped in three-dimensional, pseudopapillary, and cribriform clusters with atypical nuclei and prominent nucleoli. Intranuclear vacuoles and multinucleated cells are occasionally seen. The cytoplasm is abundant and gray blue using MGG stain (Fig. 2). Oncocytic cells with intracytoplasmic vacuoles



Ductal Carcinoma of Salivary Gland, Cytological Findings, Fig. 2 Salivary duct carcinoma. Numerous malignant and isolated cells (MGG stain)



Ductal Carcinoma of Salivary Gland, Cytological Findings, Fig. 3 Salivary duct carcinoma. Oncocytic-like cells (MGG stain)

are frequently seen (Fig. 3). Mitotic figures are present. Necrosis may be predominant (Klijanienko and Vielh 1998a).

Due to hypercellularity and the presence of atypical cells, SDC is usually easily cytologically diagnosed as adenocarcinoma.

Immunophenotype

SDC shows immunoreactivity for Her-2/*neu*, cytokeratin 7, EMA, and CEA. Androgen, estrogen, and progesterone receptors, as well as PSA, were reported to be positive in some cases.

Molecular and Cytogenetic Features

Half of the tumors overexpress p53 and tumors exhibiting overexpression of Her-2/*neu* protein may also show Her-2/*neu* gene amplification (Barnes et al. 2005; Ellis and Auclair 2008).

Differential Diagnosis

Accurate typing may be more difficult and SDC should be differentiated from other high-grade malignancies such as high-grade mucoepidermoid carcinoma (MEC), squamous cell carcinoma (SCC), oncocytic carcinoma (OC), and breast ductal cell carcinoma metastatic to the salivary gland (DIC).

High-grade MEC is composed of squamoid, intermediate, and occasionally from mucus-secreting cells. The presence of intermediate cells is in favor of MEC (Klijanienko and Vielh 1997). Primary or secondary squamous cell carcinoma is composed of round, ovoid, and polygonal malignant cells. Squamous keratin debris and squamous-like necrosis are characteristic of squamous cell carcinoma, because MEC usually does not exhibit important squamous differentiation (Klijanienko and Vielh 1998a). Malignant oncocytoma may be difficult to differentiate from SDC. Metastatic DIC from the breast is morphologically similar, and the diagnosis of SDC may be done only after clinical or radiological exclusion of the synchronous or metachronous breast adenocarcinoma (Lussier et al. 2000).

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E

Endocervical Adenocarcinoma, In Situ

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Synonyms

AIS; Cervical adenocarcinoma in situ; Endocervical intraepithelial glandular neoplasia; HG-CGIN; High-grade cervical glandular intraepithelial neoplasia

Definition

Endocervical adenocarcinoma in situ (AIS) is a precursor of endocervical adenocarcinoma, first described by Friedell and McKay in 1953 (Friedell and McKay 1953). The WHO definition of AIS is a lesion in which normally situated endocervical glands are partly or wholly replaced by cytologically malignant epithelium; by definition, there is no evidence of stromal invasion (Tavassoli and Devilee 2003). In cytological preparations, the atypical glandular cells are represented in short strips, rosettes, or crowded sheets or as scattered cells, all of which display nuclear atypia.

Clinical Features

The majority of women are asymptomatic, and the lesion is detected on a routine cervical smear. There are no definite pathognomic features of AIS on clinical or colposcopic evaluation of the cervix, as the lesion develops within the transformation zone and endocervical canal.

- **Incidence**
  AIS is much less common than its squamous counterpart, high-grade squamous intraepithelial lesion (HSIL)/high-grade cervical intraepithelial neoplasia (high-grade CIN), but is of increasing incidence. This may in part be a relative increase as a result of a reduction in squamous lesions due to organized cervical screening programs in developed countries, but there is evidence of a real increase in the prevalence of premalignant cervical glandular lesions. A study from Sweden found that the incidence of AIS increased from 0.04/100000 person-years in the 1950s and 1960s to 1.37 in the early 1990s, a proportionately greater increase incidence than that of endocervical adenocarcinoma (Hemminki et al. 2002). Approximately 50% of cases of AIS are accompanied by squamous intraepithelial lesion (SIL) or invasive squamous carcinoma (Lee and Flynn 2000).

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4

- **Age**
  The age range of AIS is similar to that of patients with HSIL, both the median and mean age being in the fourth decade, 10–15 years younger than the corresponding median and mean ages of patients with cervical adenocarcinoma.
- **Sex/Site**
  It is a disease of the endocervix in females.
- **Treatment and Outcome**
  AIS is mainly treated by excisional procedures, namely, diathermy loop excision, large loop excision of the transformation zone (LLETZ), cold knife cone biopsy, laser cone biopsy, or needle excision of the transformation zone. Hysterectomy or a repeat local excisional procedure may be performed in cases where the excision margin of an initial local excisional procedure is involved by the lesion. The treatment of choice for women who have completed childbearing is simple hysterectomy. Based on previous literature reports, it is estimated that the progression of AIS if left untreated to invasive adenocarcinoma takes about 5 years (Poynor et al. 1995).

Macroscopy

Colposcopic examination of the cervix as well as gross examination of local excisional or hysterectomy specimens does not reveal any abnormality in the majority of the cases.

Microscopy

In liquid-based cytology (LBC) samples, there are crowded (three- to four-cell thick) clusters comprised of columnar cells with oval to cigar-shaped nuclei, fine or coarse granular chromatin, occasional conspicuous nucleoli, and mitoses. Large prominent nucleoli are uncommon in AIS, compared to endocervical adenocarcinoma. Short strips of endocervical cells showing fanning out and cell palisading are characteristic of AIS. Prominent pseudostratification with nuclear overlapping at the edge of cell groups (so-called "feathering") and the presence of single cells with oval bulging nuclei giving a "snake and egg" appearance are common features of AIS. The background is usually free of necrotic cell debris but may contain neutrophil polymorphs and blood. The cytopathologist should keep in mind that a squamous abnormality can coexist with the glandular lesion in a significant number of cases.

Immunophenotyping/Molecular Features

Human papillomaviruses, especially HPV 16 and 18, have been frequently observed in endocervical adenocarcinoma and AIS lesions. HPV 18 is more frequently associated with endocervical neoplasia. The majority of AIS lesions are positive for p16 (a surrogate marker of HPV infection), bcl-2, and carcinoembryonic antigen (CEA) and negative for vimentin and estrogen receptor (ER) (Tavassoli and Devilee 2003).

Differential Diagnosis

1. Cervical smears with HSIL (high-grade squamous intraepithelial lesion)/high-grade dyskaryosis involving endocervical crypts.
   HSIL with crypt involvement shows thick clusters of dyskaryotic cells with chaotic architecture. The clusters may have flattened sharp edges. The squamous dyskaryotic cells show nuclear hyperchromasia; irregular nuclear membranes; round, oval, or angulated nuclei; coarse chromatin; and dense well-defined cytoplasm. Nuclear streaming, oval nuclei, and wispy cytoplasm of these dyskaryotic cell groups may mimic a cervical glandular lesion in LBC samples, but rosette formation, feathering, fanning out, and palisading are absent.
2. Tuboendometrioid metaplasia (TEM). TEM presents in cytological preparations as sparse groups of two- or three-dimensional crowded

epithelial cells with central hyperchromatic nuclei and minimal cytoplasm lacking the architectural features of AIS. Cilia and terminal bars may be identified.

3. Endocervicitis. In contrast to normal endocervical cells, which present as single or monolayered strips of columnar cells or flat sheets of cells resembling a honeycomb, inflamed endocervical cells present as crowded groups with dense, cyanophilic, or eosinophilic cytoplasm, and occasional cilia and terminal bars. However, there is maintenance of intercellular spacing and none of the architectural features of AIS.
4. Endometrial cells. During menstruation and in the early part of the proliferative phase, endometrial cells form tight three-dimensional clusters consisting of a central core of compact stromal cells surrounded by a peripheral rim of loose dark epithelial cells, architecturally different from AIS. In the later phases of the menstrual cycle, the cells undergo degenerative change and form compact groups of dark-staining small cells which may be associated with histiocytes and neutrophil polymorphs. An awareness of these appearances, coupled with information as to the timing of the smear sample, is important in making the correct differential diagnosis.
5. Lower uterine segment sampling. Sampling devices may reach high into the endocervical canal and directly sample cells from the lower uterine segment. These present in cytological preparations as poorly cohesive cuboidal cells with delicate vesicular nuclei lacking the architectural features of AIS. In some cases, these cells are associated with stromal cells and form characteristic tubular microbiopsies with peripheral palisading and a surface layer of elongated stromal cells. Capillary vascular spaces may be identified within the stromal component.
6. Endometriosis. This is characterized in cytological preparations by the finding of endometrial cells in strips or sheets and endometrial stromal cells in loose groups which may be admixed with the epithelial cells. Samples are often heavily bloodstained and may contain degenerate red blood cells.

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Endocervical Adenocarcinoma, Invasive

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Definition

Invasive endocervical adenocarcinomas are invasive epithelial tumors of the endocervix characterized by glandular differentiation. They are categorized into different subtypes as per the 2014 World Health Organization classification – usual, mucinous, villoglandular, clear cell, serous, and mesonephric types. The usual type is the most common type of endocervical adenocarcinoma.

Clinical Features

- **Incidence**
  Though there has been a decline in the overall incidence of cervical cancer in the United States, there has been an increase in the incidence of endocervical adenocarcinomas. As per the Surveillance Epidemiology and End Results database, endocervical adenocarcinomas accounted for 24.2% of cervical carcinomas from 1990–2008 and 14% of cervical carcinomas from 1973–1989. The expected annual percentage change for endocervical adenocarcinomas is reported to be 0.6%. This increase in incidence is at least in part due to better endocervical sampling devices, better cytologic preparations, and enhanced cytologic recognition of glandular preinvasive and invasive lesions.
- **Age**
  The mean age at presentation for endocervical adenocarcinomas is 50 years which is around 10–15 years more than the average age for adenocarcinoma in situ (AIS).
- **Site**
  Most of the endocervical adenocarcinomas arise in the transformation zone. They may also occur higher up in the endocervical canal.
- **Treatment**
  The treatment for endocervical adenocarcinomas depends upon the stage of the disease. For stage IA1 cancers that do not show lymphovascular invasion, a simple hysterectomy or a cold knife conization can be performed. For higher stages (IA2, IB, and IIA) a radical hysterectomy with lymph node dissection (pelvic nodes with or without para-aortic nodes) is preferred. For advanced stages (IIB and above), treatment involves chemoradiation.
- **Outcome**
  The prognosis for endocervical adenocarcinomas depends upon the stage. However, gastric type of adenocarcinomas has worse prognosis as compared to the usual type – they show a 30% 5-year disease-free survival as compared to 74% for the usual type. The villoglandular variant, if superficially invasive with no lymphovascular invasion, has an excellent prognosis.

Macroscopy

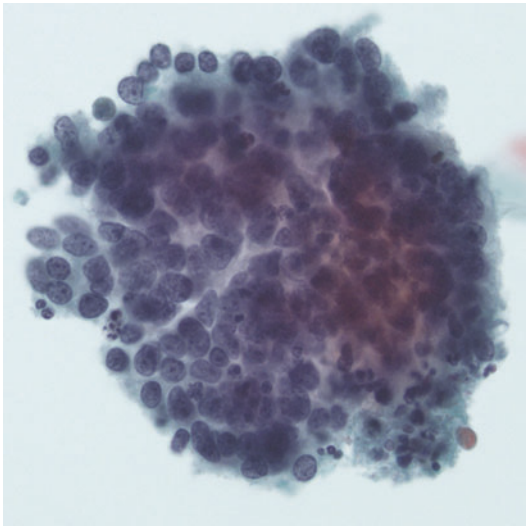
Many endocervical adenocarcinomas will form a fungating mass with polypoid or papillary features. In others, the cervix may show surface ulceration or diffuse enlargement resulting in a "barrel shaped" appearance. In some cases, no lesions may be grossly apparent.

Microscopy

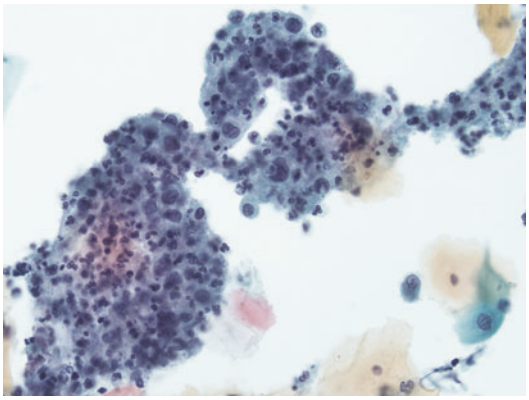
- **Usual type**
  The morphologic features overlap with those of AIS with more pronounced architectural and cytologic abnormalities. The cells can be seen in two-dimensional sheets, three dimensional clusters, or syncytial groups. The cytoplasm is usually finely vacuolated. The nuclei show irregular chromatin distribution with clearing of chromatin. The nuclear membrane can be irregular and nucleoli tend to be very prominent (macronucleoli). Tumor diathesis is usually visible as debris on the periphery of the cell clusters – "clinging diathesis" (Figs. 1, 2, and 3).
- **Mucinous (gastric) type**
  The gastric type of mucinous carcinomas can be very challenging to recognize on cytology. They usually exhibit two-dimensional arrangements and have low nuclear cytoplasmic ratio. The cytoplasm may be mucinous and show a yellowish tinge. Alternatively, it may be vacuolar and/or foamy. Intracytoplasmic neutrophil entrapment may be seen. The nuclei are typically vesicular and nucleoli can be prominent. The background may show mucin (Figs. 4 and 5).
- **Villoglandular type**
  They show branching tissue fragments or bulbous groups with nuclear crowding and minimal cytologic atypia.

Immunophenotype

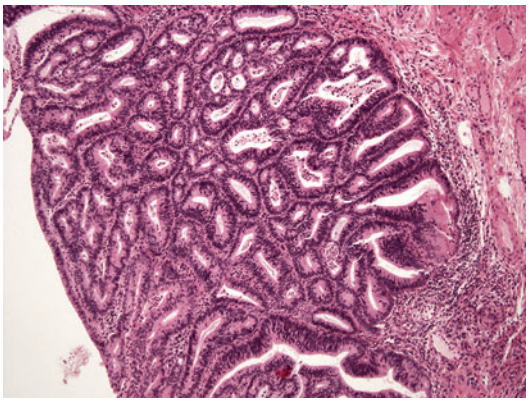
The usual type of endocervical adenocarcinomas show strong and diffuse expression of p16 and tend to have loss of ER (estrogen receptor) and PR (progesterone receptor). The gastric type of



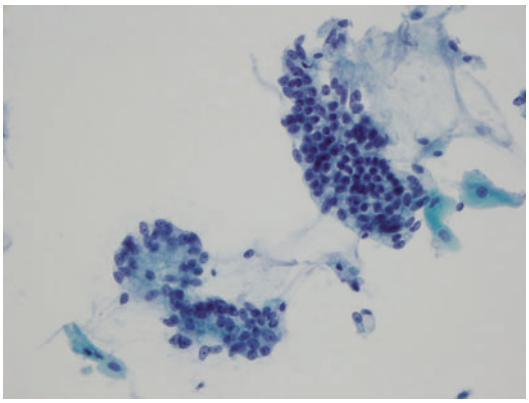
Endocervical Adenocarcinoma, Invasive, Fig. 1 Endocervical adenocarcinoma – tumor cells show nuclear crowding and irregular chromatin distribution with "clinging" diathesis (*bottom right*) (ThinPrep, Papanicolaou stain, 400×)



Endocervical Adenocarcinoma, Invasive, Fig. 2 Endocervical adenocarcinoma – tumor cells with irregular nuclear membranes in a background of tumor diathesis (ThinPrep, Papanicolaou stain, 400×)



Endocervical Adenocarcinoma, Invasive, Fig. 3 Invasive endocervical adenocarcinoma with cribriform architecture (H&E stain, 400×)

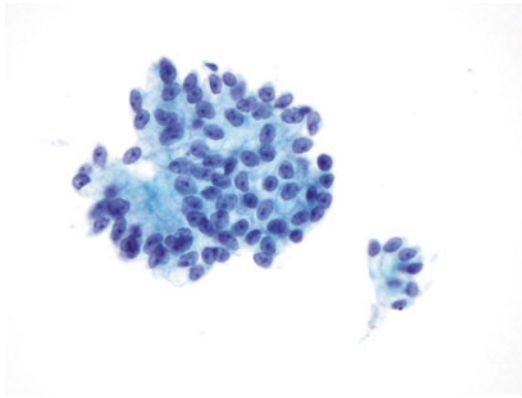


Endocervical Adenocarcinoma, Invasive, Fig. 4 Gastric type mucinous adenocarcinoma – cells show a two-dimensional architecture and abundant mucinous cytoplasm in a background of thick mucin (ThinPrep, Papanicolaou stain, 400×)

mucinous carcinomas is positive for MUC6 and is usually negative for ER, PR, and p16.

Molecular Features

Majority (>90%) of invasive endocervical adenocarcinomas and almost 100% of AIS are etiologically related to high-risk HPV infection. The most common HPV types are types 18, 16, and 45. Some studies have reported HPV 18 to be more commonly associated with endocervical adenocarcinomas. The integration of high-risk HPV DNA into the host genome results in the overexpression of E6 and E7 viral oncogenes which inhibit the tumor suppressor functions of p53 and pRb proteins, respectively, thereby resulting in carcinogenesis. The gastric type of mucinous carcinomas commonly show STK11



Endocervical Adenocarcinoma, Invasive, Fig. 5 Gastric type mucinous adenocarcinoma – monotonous appearing tumor cells with prominent nucleoli (ThinPrep, Papanicolaou stain, 600×)

mutations (chromosome 19p) and can be associated with Peutz-Jeghers syndrome.

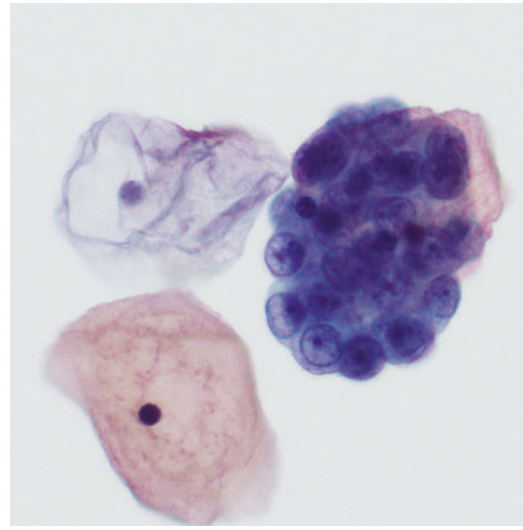
Differential Diagnosis

AIS – Invasive endocervical adenocarcinomas show more marked architectural crowding and cytologic atypia as compared to AIS. Also, presence of tumor diathesis helps in the distinction between AIS and adenocarcinoma.

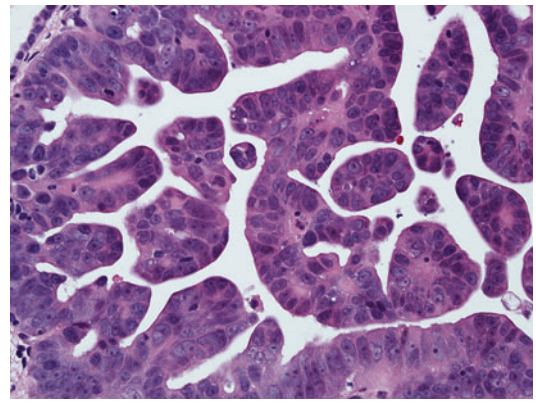
Non-keratinizing squamous cell carcinomas – The presence of focal keratinization can help in recognizing squamous differentiation and in the distinction of nonkeratinizing squamous cell carcinomas from endocervical adenocarcinomas.

Endometrial adenocarcinomas – These are seen in three-dimensional groups as compared to endocervical adenocarcinomas which can occur in two-dimensional sheets with columnar configuration (Figs. 6 and 7). Also, in an older patient, the adenocarcinoma is more likely to be endometrial in origin.

Reactive endocervical glandular cells – The morphologic features of reactive endocervical glandular cells can overlap with those of endocervical adenocarcinomas. They form flat sheets and show finely distributed chromatin with smooth nuclear membranes and visible to prominent nucleoli.



Endocervical Adenocarcinoma, Invasive, Fig. 6 Endometrial adenocarcinoma – the cells are seen in crowded three-dimensional groups with nuclear enlargement and prominent nucleoli (ThinPrep, Papanicolaou stain, 400×)



Endocervical Adenocarcinoma, Invasive, Fig. 7 Serous adenocarcinoma of endometrium (H&E stain, 400×)

References and Further Reading

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Endometrial Cells, Smears

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Definition

The finding of benign-appearing exfoliated endometrial cells in a Pap test may be of clinical significance, especially in postmenopausal women. As per the 2001 Bethesda System, the age for reporting these cells was ≥40 years. However, the studies that followed revealed a low risk of significant pathology (endometrial hyperplasia or adenocarcinoma) associated with this finding. Hence, the 2014 Bethesda System has increased the reporting age of benign-appearing endometrial cells in Pap tests to ≥45 years to enhance their predictive value for endometrial neoplasia. This finding falls under the "Other" category of the 2014 Bethesda System.

Abraded endometrial cells from the lower uterine segment, histiocytes, and stromal cells should not be reported as they are not clinically significant.

Clinical Features

The presence of endometrial cells in cervical cytology can be asymptomatic. Some women may have risk factors for endometrial cancer such as obesity, polycystic ovarian syndrome, history of tamoxifen use, diabetes, and hypertension. Postmenopausal women may exhibit symptoms associated with the finding of endometrial cells. Mostly, they present with vaginal bleeding.

- **Incidence**
  Following the implementation of the Bethesda System 2001, there was increase in the incidence of endometrial cells in Pap tests to 0.49–0.61% due to the reporting of this finding in premenopausal women as well. Endometrial cells are more commonly seen in the first half of the menstrual cycle as compared to the second half. They are detected more frequently in premenopausal than in postmenopausal women. In older women (≥40 years or postmenopausal), the frequency of benign-appearing endometrial cells ranges from 0.4% to 3% of Pap tests.
- **Age**
  Benign-appearing endometrial cells can be seen in Pap tests of women of reproductive age group normally during menstruation and the proliferative phase. In premenopausal women, they can be seen normally up to day 12 of the cycle. However, in postmenopausal women, their presence may be an indication of underlying endometrial neoplasia.
- **Treatment**
  Exfoliated benign-appearing endometrial cells in premenopausal women need not be evaluated further unless clinically indicated. However, endometrial sampling is recommended in postmenopausal women regardless of symptoms.
- **Outcome**
  Benign-appearing endometrial cells in the Pap test can be seen with conditions such as polyps, endometrial hyperplasia with and without atypia, endometrial adenocarcinoma (usually endometrioid type), leiomyomata, atrophy, proliferative endometrium, immediate postpartum state, impending or early postabortion, acute endometritis, recent intrauterine instrumentation, IUD use, and cervical and vaginal endometriosis. The risk of significant

pathology in women ≥ 40 years is low – endometrial hyperplasia (2%) and carcinoma (1.1%). However, the risk for significant pathology is higher for postmenopausal women – 7% to 12% as per various studies.

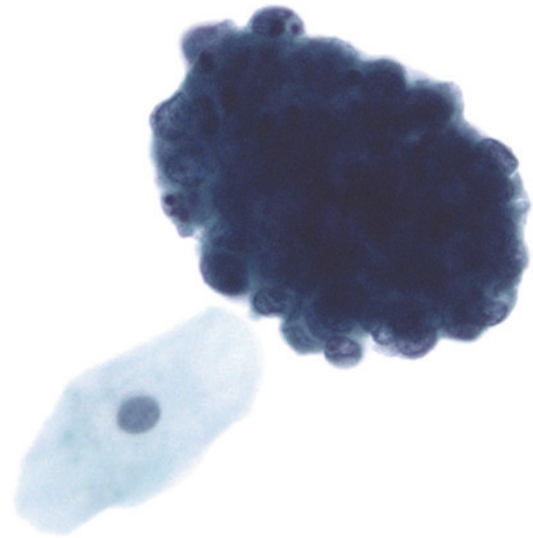
Macroscopy

Some women with the finding of endometrial cells on cervical cytology may have thickened endometrium or endometrial lesions such as polyp on ultrasound.

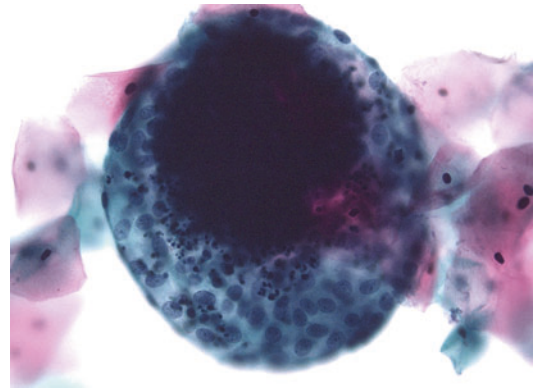
Microscopy

Benign-appearing endometrial cells are usually seen in tight three-dimensional clusters. The cells are small with scant cytoplasm that may be vacuolated. The nuclear size approximates that of an intermediate cell nucleus. The nuclei are hyperchromatic with inconspicuous nucleoli (Fig. 1). Apoptotic bodies are easily seen. Liquid-based preparations can show singly lying endometrial cells and enhanced chromatin detail. Double-contoured aggregates of stromal cells and epithelial cells, referred to as "exodus," are common during days 6–10 of the cycle. They are composed of a hyperchromatic core of stromal cells with apoptotic bodies surrounded by an outer envelope of endometrial glandular cells (Fig. 2). Histologically, these correlate with stromal breakdown.

Two types of endometrial stromal cells can be identified in the Pap test – superficial and deep stromal cells. The superficial stromal cells resemble histiocytes with more cytoplasm and oval or bean-shaped nuclei. The deep stromal cells have scant cytoplasm with ovoid or spindle-shaped nuclei (Fig. 3).



Endometrial Cells, Smears, Fig. 1 Endometrial cells – three-dimensional groups with scant cytoplasm and nuclear size approximating that of an intermediate cell nucleus (ThinPrep, Papanicolaou stain, 400×)

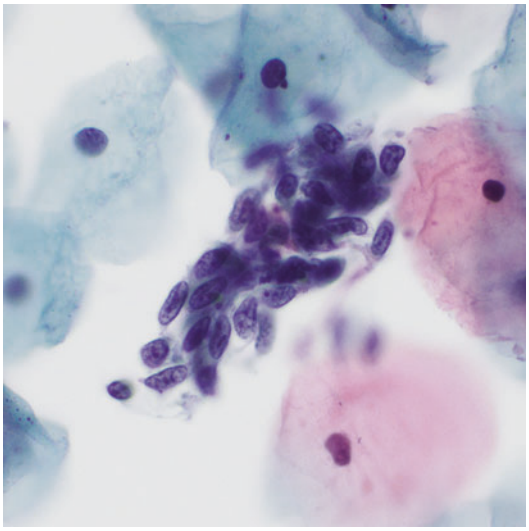


Endometrial Cells, Smears, Fig. 2 Exodus – double-contoured groups with stromal cells in the center with apoptotic bodies, surrounded by epithelial cells (ThinPrep, Papanicolaou stain, 400×)

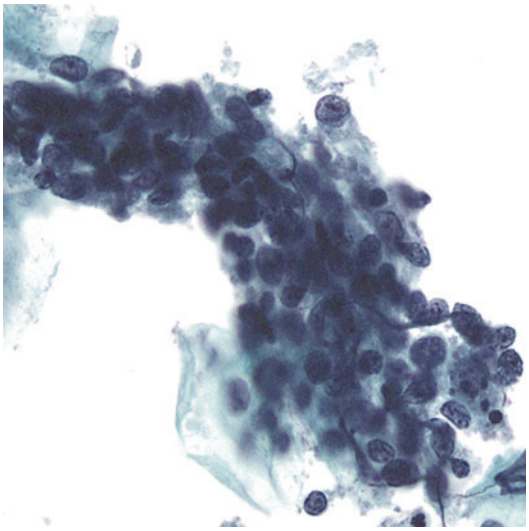
Differential Diagnosis

Atypical endometrial cells – In contrast to benign-appearing endometrial cells, atypical endometrial cells primarily show enlarged nuclei. The nucleus is enlarged in comparison to an intermediate cell nucleus. Other features that may be seen include small or prominent nucleoli, heterogeneous chromatin, and presence of intracytoplasmic neutrophils.

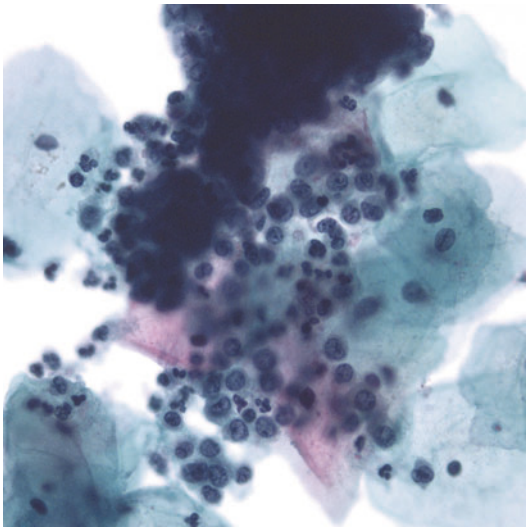
Histiocytes – These can occur singly or in loose aggregates and may be seen in association with endometrial cells. They have moderate amounts of pale cytoplasm with reniform,



Endometrial Cells, Smears, Fig. 3 Endometrial deep stromal cells (ThinPrep, Papanicolaou stain, 400×)



Endometrial Cells, Smears, Fig. 5 Follicular cervicitis – polymorphous population of lymphocytes with tingible-body macrophages (ThinPrep, Papanicolaou stain, 400×)



Endometrial Cells, Smears, Fig. 4 Histiocytes with moderate amounts of cytoplasm, ovoid to reniform nuclei, and fine chromatin (ThinPrep, Papanicolaou stain, 400×)

grooved, or folded nuclei and finely distributed chromatin (Fig. 4).

High grade squamous intraepithelial lesion (HSIL) – Singly lying endometrial cells may be confused with HSIL cells. The morphologic similarity of these cells to other easily recognizable groups of endometrial cells can help in their characterization.

Endocervical cells – Endocervical cells have a flat arrangement of cells in comparison to endometrial cells. They are columnar in shape and have more amounts of delicate cytoplasm with vesicular chromatin.

Lower uterine segment endometrium (LUS) – Abraded LUS can be seen due to inadvertent sampling of the lower uterine segment especially in patients who have undergone an excisional procedure such as LEEP, cone biopsy, or a trachelectomy. The fragments tend to be biphasic being composed of well-organized endometrial cells in sheets or branching tubules and closely associated stromal cells with bland nuclear chromatin. However, these two components are usually seen separately in liquid-based preparations.

Follicular cervicitis – Follicular cervicitis is characterized by the finding of clusters of lymphoid cells with tingible-body macrophages. The lymphocytes show a coarse chromatin distribution as compared to the endometrial cells (Fig. 5).

Small cell carcinoma – Small cell carcinoma cells can be very challenging to distinguish from

endometrial cell. The cells of small cell carcinoma tend be darker and also exhibit crush artifact and nuclear molding.

Clusters of naked nuclei – These clusters of naked nuclei are typically seen in atrophic specimens. They are devoid of cytoplasm and show bland chromatin. They are possibly parabasal or reserve cell in origin and can mimic endometrial cells.

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Endometrioid Ovarian Tumors, Cytological Findings

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Synonyms

Endometrioid ovarian neoplasms

Definition

According to WHO Histological Classification of Tumours of the Ovary, these tumors belong to surface epithelial-stromal ovarian tumors, and are named as Endometrioid Tumors Including Variants with Squamous Differentiation (Tavassoli and Devilee 2003). They are divided into malignant, borderline, and benign tumors. Malignant tumors are adenocarcinoma not otherwise specified, adenocarcinofibroma (malignant adenofibroma), malignant Müllerian mixed tumor (carcinosarcoma), adenosarcoma, endometrioid stromal sarcoma (low grade), and undifferentiated ovarian sarcoma. Borderline tumors are cystic tumor, and adenofibroma and cystadenofibroma. Benign tumors are cystadenoma, and adenofibroma and cystadenofibroma (Tavassoli and Devilee 2003). Endometrioid ovarian tumors closely resemble endometrioid tumors of the uterine corpus. They are often associated with endometriosis of the same ovary and/or other sites, and in some cases, they are present in the endometriotic cyst, that indicates their origin from endometriosis (Tavassoli and Devilee 2003). In other cases, they can be present together with endometrial tumor, that indicates the same risk factors (Tavassoli and Devilee 2003). The vast majority of endometrioid ovarian tumors are carcinomas (Kurman et al. 2011).

Clinical Features

- **Incidence**
  Endometrioid ovarian carcinomas account for 10–20% of ovarian carcinomas (Tavassoli and Devilee 2003).
- **Age**
  Endometrioid ovarian carcinomas occur most commonly in the fifth and sixth decades of life. The patients with preexisting endometriosis are 5–10 years younger on average than patients without endometriosis (Tavassoli and Devilee 2003).

Macroscopy

Endometrioid carcinomas have a smooth outer surface. On cut section, they are solid and

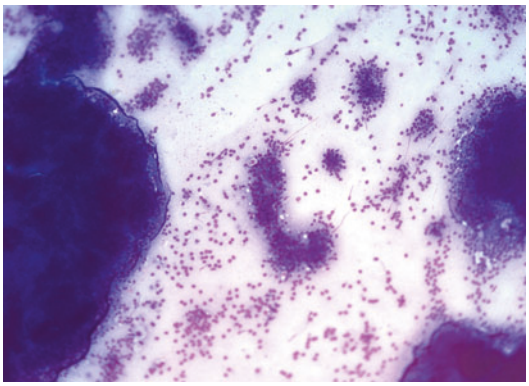
cystic. Carcinomas arising in endometriosis look like endometriotic cyst with tumor protruding from the cyst wall (Kurman et al. 2011).

Microscopy

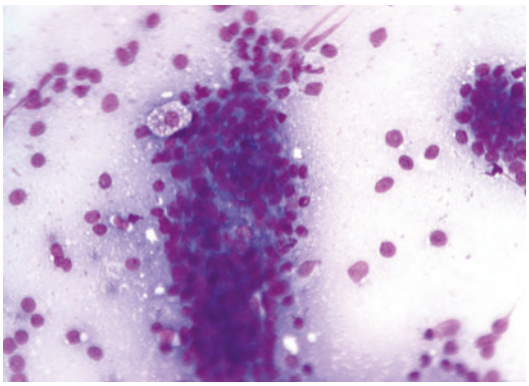
Imprint

Endometrioid ovarian adenocarcinoma can be well, moderately, or poorly differentiated. The majority are well differentiated (Kurman et al. 2011). Cytological findings in tumor imprints show high cellularity and apparent glandular pattern resembling endometrial glands. The overcrowded tubular glands, some with branching, bigger and smaller cell groups, single malignant epithelial cells, scattered stromal cells, and occasional foam cells, are found (Figs. 1–3). The overlapping tumor cells in glandular formations show weak cohesion and disturbed polarity. Peripheral dissociation of cells in the groups is typical (Jiménez-Ayala and Jiménez-Ayala Portillo 2011). Peripherally tumor cells are often loosely arranged in palisades and some cells drop off the cluster (Figs. 4 and 5). The tumor cells are moderately enlarged if compared with endometrial cells, and rather uniform. The nuclei are round to oval, hyperchromatic, eccentric in position and have finely granular irregularly distributed chromatin with prominent nucleoli (Manek and Mahovlić 2010). The cytoplasms are scant, unclearly outlined, homogeneous to finely granular, or sometimes vacuolated (Jiménez-Ayala and Jiménez-Ayala Portillo 2011; Manek and Mahovlić 2010). Psammoma bodies are occasionally found. Moderately differentiated ovarian endometrioid adenocarcinoma shows more pronounced cell dissociation and anisocytosis (Figs. 6 and 7), while in poorly differentiated endometrioid adenocarcinoma, the features of malignancy are prominent and the endometrioid cell type is difficult to assess, namely, a lot of single cells are present together with glandular formations of loosely arranged cells.

Squamous differentiation is present in 30–50% of endometrioid ovarian adenocarcinomas (Tavassoli and Devilee 2003). Usually the squamous cells form morula, the round aggregate of cells, that can be benign, or keratinized and atypical (Tavassoli and Devilee 2003). In cytological specimens, squamous cells are mixed with other cells.



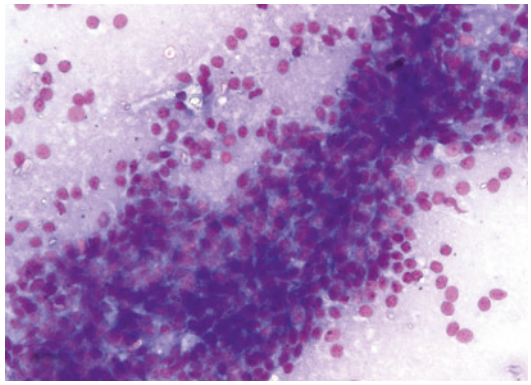
Endometrioid Ovarian Tumors, Cytological Findings, Fig. 1 Well-differentiated endometrioid ovarian adenocarcinoma. Endometrial-like glands, occasional foam cell (Imprint, MGG ×100)



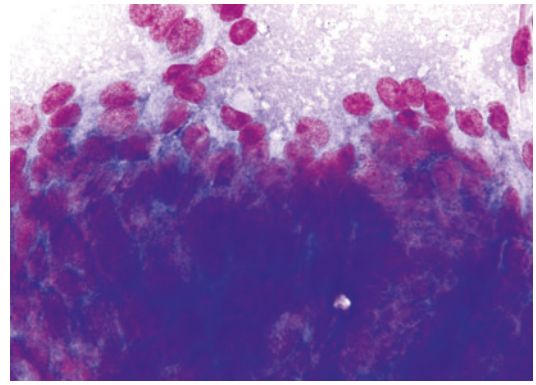
Endometrioid Ovarian Tumors, Cytological Findings, Fig. 2 Well-differentiated endometrioid ovarian adenocarcinoma. Endometrial-like gland, occasional foam cell (Imprint, MGG ×400)

Fine Needle Aspiration (FNA)

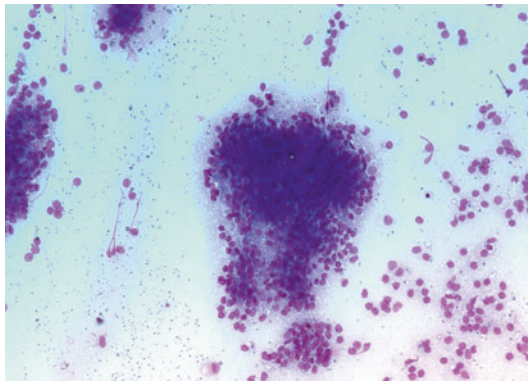
The cytological finding reveals tumor cells in three-dimensional acinar arrangements, papillary formations, smaller groups and single cells, with histiocytes, leukocytes, erythrocytes, stromal cells, and sometimes necrosis (Jiménez-Ayala



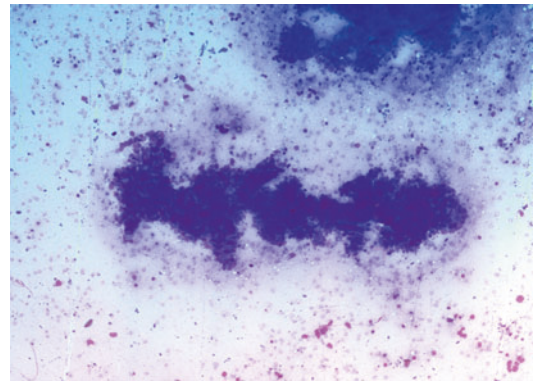
Endometrioid Ovarian Tumors, Cytological Findings, Fig. 3 Well-differentiated endometrioid ovarian adenocarcinoma. Glandular endometrial-like pattern (Imprint, MGG ×400)



Endometrioid Ovarian Tumors, Cytological Findings, Fig. 5 Well-differentiated endometrioid ovarian adenocarcinoma. Peripheral dissociation of cells in the group (Imprint, MGG ×1000)



Endometrioid Ovarian Tumors, Cytological Findings, Fig. 4 Well-differentiated endometrioid ovarian adenocarcinoma. Peripheral dissociation of cells in the group (Imprint, MGG ×200)



Endometrioid Ovarian Tumors, Cytological Findings, Fig. 6 Moderately differentiated endometrioid ovarian adenocarcinoma. Tubular glandular structure with branching (Imprint, MGG ×100)

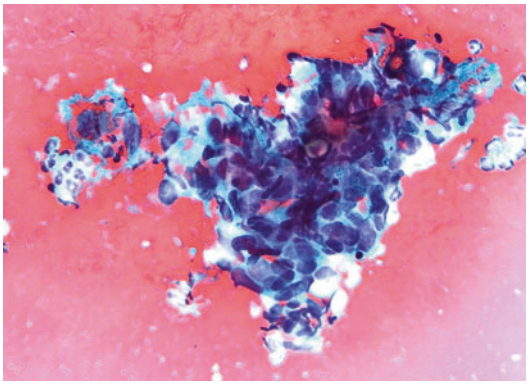
and Jiménez-Ayala Portillo 2011; Manek and Mahovlić 2010).

Peritoneal Washing and Ascites

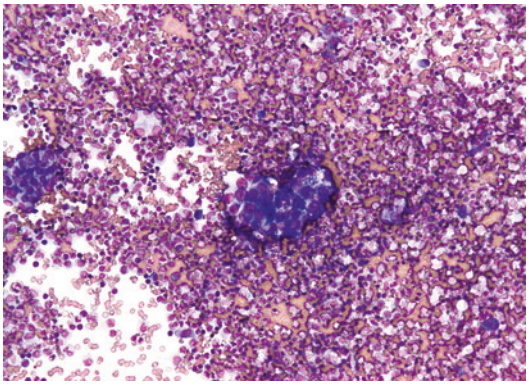
The cytological finding reveals tumor cells mostly in rounded cell groups and glandular formations, and some cells are enlarged with large single vacuole pushing the nucleus peripherally (Fig. 8). The tumor cells in ascites are broken off the peritoneal lining and they float in fluid, showing rounded cell clusters and cytoplasmic vacuolization more pronounced than in tumor imprints and aspirates (Figs. 9 and 10).

Other Endometrioid Ovarian Tumors: Cytological Findings

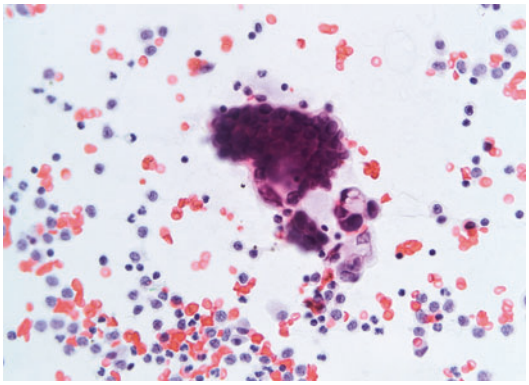
Malignant ovarian Müllerian tumor (carcinosarcoma) is composed of two malignant components, epithelial and stromal, and both of them can be found in cytological specimens, mostly comprising malignant epithelial component as adenocarcinoma, while sarcomatous component presents with isolated or small groups of malignant spindle cells, or as single large cells with bizarre configurations, dense cytoplasm, and markedly abnormal nuclei (Manek and Mahovlić 2010).



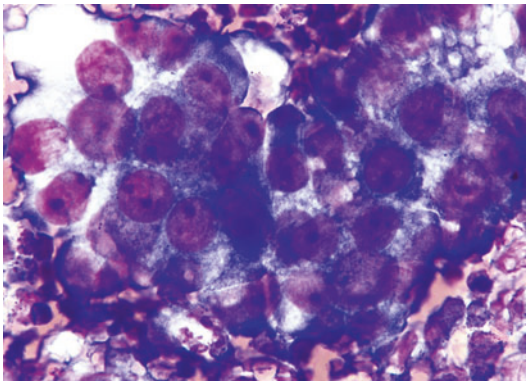
Endometrioid Ovarian Tumors, Cytological Findings, Fig. 7 Moderately differentiated endometrioid ovarian adenocarcinoma. Three-dimensional cell cluster (Peritoneal washing, Papanicolaou ×400)



Endometrioid Ovarian Tumors, Cytological Findings, Fig. 9 Poorly differentiated ovarian endometrioid adenocarcinoma. Acinar formation among numerous inflammation cells and erythrocytes (Ascites, MGG ×200)



Endometrioid Ovarian Tumors, Cytological Findings, Fig. 8 Poorly differentiated endometrioid ovarian adenocarcinoma. The cytological finding is similar to serous adenocarcinoma (Ascites, Papanicolaou ×400)



Endometrioid Ovarian Tumors, Cytological Findings, Fig. 10 Poorly differentiated endometrioid ovarian adenocarcinoma. Acinar formation of cells with marked features of malignancy and cytoplasmic vacuolization (Ascites, MGG ×1000)

Ovarian adenosarcoma is a biphasic tumor resembling the uterine adenosarcoma, with endometrioid epithelial glandular structures, mostly benign, occasionally atypical, and embedded in malignant stromal component (Tavassoli and Devilee 2003). Cytological findings of this tumor are seldom described. In a reported case, few malignant stromal cells were found in ascitic fluid, as dispersed tumor cells with large cytoplasms, while nuclei were oval-shaped, with nuclear invagination, finely granular chromatin, and one to two conspicuous nucleoli (Hirakawa et al. 2001).

Endometrioid benign ovarian tumors are rare. Cytological findings of **endometrioid ovarian adenofibroma** (fine needle aspiration and tumor tissue imprint) show benign endometrial-like epithelial cells and spindle stromal cells. In contrast to endometriosis, mitoses are rarely seen in the epithelial component, while the absence of endometrial-type stroma and hemosiderin-laden macrophages further distinguish adenofibroma from endometriosis (Volmar et al. 2004).

Immunophenotype

Endometrioid ovarian tumors share the immunophenotype with other ovarian carcinoma subtypes (serous, clear cell, and mucinous). They are positive for cytokeratin (CK) 7 and CA125 (two relatively specific markers for ovarian epithelial tumors), BerEP4, epithelial membrane antigen, B72.3, estrogen and progesterone receptors, vimentin, and negative for inhibin and steroidogenic factor-1 (SF-1) that are markers for sex-cord tumors (Tavassoli and Devilee 2003; Manek and Mahovlić 2010).

Differential Diagnosis

Well-differentiated endometrioid adenocarcinoma can be similar to atypical hyperplasia in endometriosis, and to borderline endometrioid tumors. On the other hand, it can be similar to other malignant tumors, mostly to serous adenocarcinoma. Moderately and especially poorly differentiated endometrioid adenocarcinomas are difficult to distinguish from serous adenocarcinomas (Jiménez-Ayala and Jiménez-Ayala Portillo 2011; Manek and Mahovlić 2010).

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Endometriosis, Cytological Findings

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Synonyms

Endometrioma; Endometriosis

Definition

Endometriosis is a condition of implant of a focus of benign endometrial cells outside of the corpus (body) uterus. The etiology and pathogenesis of endometriosis is still not well understood. It usually results from retrograde menstruation and persists and may become functional in women with some form of immune dysfunction such as upregulation of inflammatory pathways that are normally deployed in response to infection or trauma. Genetics is being considered as a risk factor in the development of endometriosis and endometriosis-associated ovarian carcinomas, but the exact cellular and molecular pathways are not well elucidated.

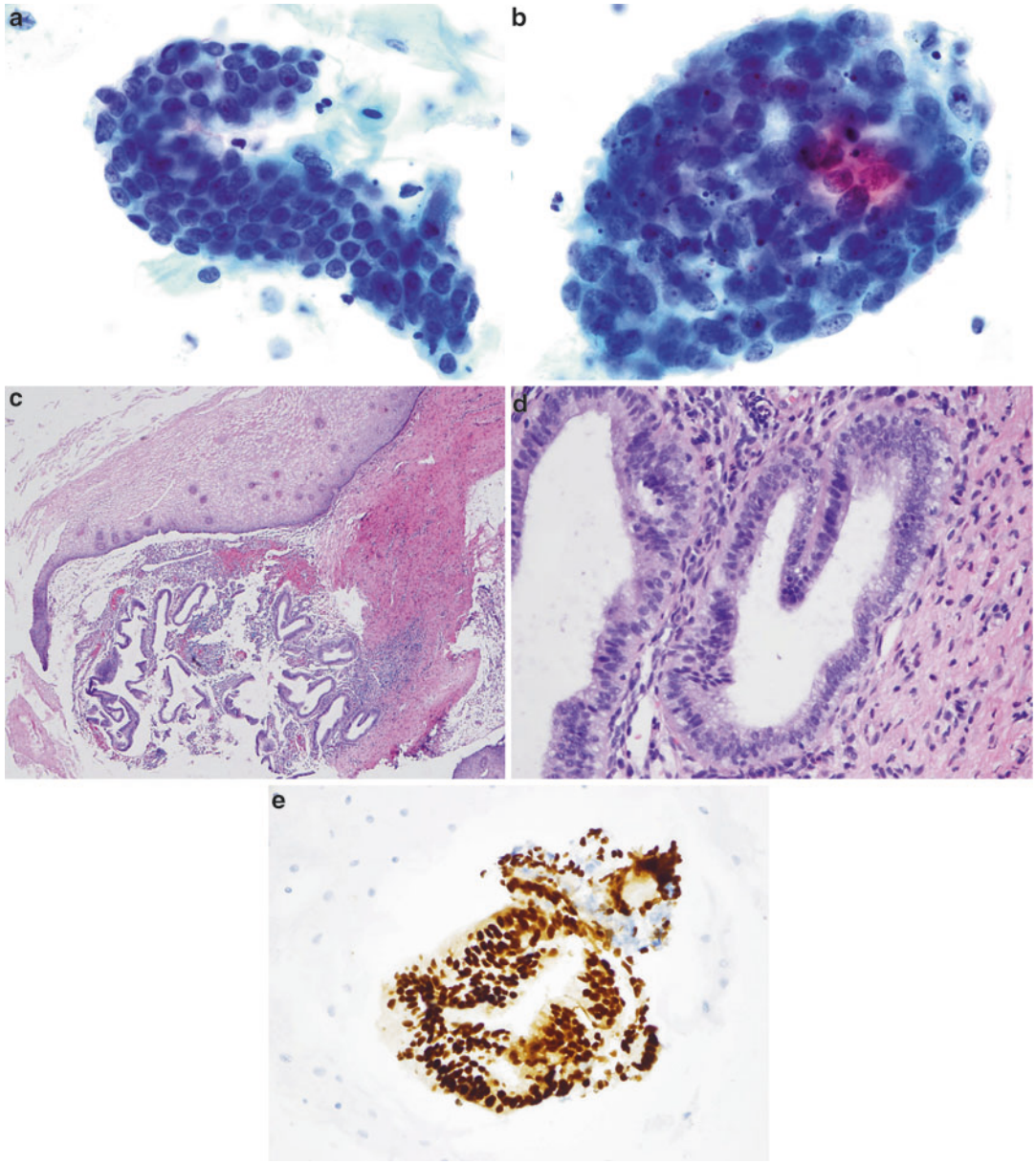
Clinical Features

- **Incidence**
  The exact incidence of endometriosis is difficult to estimate, as some lesions are asymptomatic. Current estimates suggest that 6–10% of women of reproductive age, >50% of women and teenage girls with pelvic pain, and 50% of women with infertility have endometriosis. In the United States, ~5 million women, mostly in their 30s or 40s, have the

disease. Risk factors for endometriosis include obstruction to menstrual outflow (mullerian anomalies), exposure to diethylstilbestrol in utero, prolonged exposure to endogenous estrogens (early menarche, late menopause, or obesity), short monthly menstrual cycle, heavy menstrual cycles, and genetic predilection (mother, sister, or daughter with endometriosis). Endometriosis is common among women suffering from infertility. Women with endometriosis commonly have coexisting conditions including allergies, autoimmune disorders, eczema, and asthma. Factors that lower the risk of endometriosis include prolonged lactation, multiple pregnancies, regular exercise, and diet rich in fruits and green vegetables.

Patients with high-grade squamous intraepithelial lesion (HSIL/CIN3) and glandular lesions such as endocervical adenocarcinoma in situ (AIS) are predisposed to cervical and vaginal mucosal endometriosis. Endometriosis in such patients may result from cervical trauma, following excisional procedures such as loop electrocautery excision procedures (LEEP) or cone biopsy performed for diagnosis or treatment. Cytologists need to be aware that the follow-up Pap tests for HSIL and AIS may show both recurrent neoplastic disease and endometriosis.

- **Site**
  Endometriosis most commonly involves pelvic peritoneum, ovary, rectovaginal septum, and rarely diaphragm, pleura, or pericardium. Distant sites of involvement may result from lymphatic or hematogenous spread or metaplastic transformation. Cervix and vaginal involvement may be seen following excisional procedures such as LEEP or cone biopsy.
- **Symptoms**
  Endometriosis is a cause for pelvic pain, worsening dysmenorrhea, and dyspareunia and infertility. The pelvic pain is cyclic and can occur intermittently throughout the menstrual cycle or it can be continuous. It can be dull, throbbing, or sharp and increased by physical activity. Bladder- and bowel-associated symptoms include nausea, distention, and early satiety. Menstrual periods are usually regular. Endometriosis of the cervix may occasionally cause postcoital bleeding or rarely massive vaginal bleeding (Fig. 1).
- **Genetics**
  Patients with endometriosis are significantly more likely to have a family history of endometriosis. However, the genetic mechanisms involved in familial predisposition are not well elucidated. Recent molecular and pathological evidence suggests that ovarian endometrioma is monoclonal and thus a neoplastic disease. Data also reveals that ovarian endometriosis may serve as a precursor of ovarian cancer, especially of the endometrioid and clear cell subtypes. This phenomenon is rare and occurs in 0.7–2.5% of the cases. Pathological studies have detected a morphological continuum of sequential steps from normal endometriotic cyst epithelium to atypical endometriosis and finally to invasive carcinoma. Although a variety of molecular events, such as PTEN and ARID1A mutations and upregulation of HNF-1, have been identified in endometriosis-associated carcinoma, its precise carcinogenic mechanism remains poorly understood. Current data indicate that microenvironmental factors, including oxidative stress and inflammation, play an important role.
- **Diagnosis**
  Transvaginal ultrasound and MRI are useful in detecting ovarian endometriomas. These modalities, however, are not useful for detecting smaller foci of the lesion. The definitive method to diagnose and stage endometriosis and evaluate the recurrence of disease after treatment is visualization at surgery.
- **Treatment**
  Treatments are to relieve the complications of infertility and/or pain commonly associated with endometriosis. They include hormonal therapy, pain medication, and laproscopic and surgical procedures. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to relieve dysmenorrhea. Treatment may last for years or until symptoms are relieved or the disease recurs. Surgical approaches to



Endometriosis, Cytological Findings, Fig. 1 (**a**) Case of cervical endometriosis in a 46-year-old patient who presented with vaginal bleeding. It shows a two-dimensional tubular fragment comprised of small endometrial cells with high nuclear to cytoplasmic ratio. The nuclei are small (as big as intermediate squamous cell nuclei seen at the 6 o'clock position), uniform, round to oval, with small nucleoli and scant cytoplasm; (**b**) Shows a cluster of endometrial epithelial and stromal cells. The stromal cell nuclei are oval and pale. Numerous apoptotic bodies are also noted. Hemosiderin-laden macrophages were not evident. This was reported as benign endometrial cells in a woman $\geq$45; (**c** and **d**) Cervical endometriosis. Excision was performed after endometrial cells were reported on the Pap test (H&E low and high magnification); (**e**) Immunostain for progesterone receptor was positive (high magnification)

relieve endometriosis-related pain can be used as first-line therapy or initiated after failed medical therapies. The procedures include excision, fulguration, or laser ablation of endometriotic implants on the peritoneum, excision, drainage or ablation of endometriomas, resection of rectovaginal nodules, lysis of adhesions, and interruption of nerve pathways. Ablation of endometriotic lesions with lysis of adhesions is recommended for the treatment of infertility. For cervical endometriosis, the vast majority of patients are managed conservatively unless there are significant symptoms. Patients with suspected cervical endometriosis should be investigated to confirm the diagnosis and exclude more serious lesions.

Further genetic studies to determine the cellular and molecular mechanisms involved in the pathogenesis of endometriosis need to be performed in well-characterized families to discover new drug targets for better treatment.

- **Outcome**
  In most cases, hormonal therapy, pain management, surgical intervention, and IVF treatments will give women significant relief from pelvic pain and assist them in achieving pregnancy. Follow-up of women with pelvic pain and laparoscopically identified disease has shown that 17–29% of the lesions resolve spontaneously and 20–40% may recur within 5 years of surgical intervention. Long-term treatment of patients with chronic pelvic pain associated with endometriosis involves repeated courses of medical therapy, surgical therapy, or both.

Macroscopy

"Implants," "nodules," or "patches" of areas of endometriosis are usually located on the ovaries, fallopian tubes, uterus, and parametrium or pelvic peritoneum. Such lesions may also involve the rectosigmoid, urinary bladder, and ureters and less commonly in remote extraperitoneal areas including the lung. The endometriosis lesions may vary in size and can appear dark bluish-red, blackish, yellowish-brown, or nonpigmented. Larger lesions may be seen within the ovaries as endometriomas or "chocolate cysts," "chocolate" because they contain a thick brownish fluid, mostly old blood. Not all endometriosis lesions are visible. ▶ Endometriosis in the cervix may be apparent as bluish-red or bluish-black lesions 1–3 mm in diameter, and rarely it may present as a mass (Fig. 2).

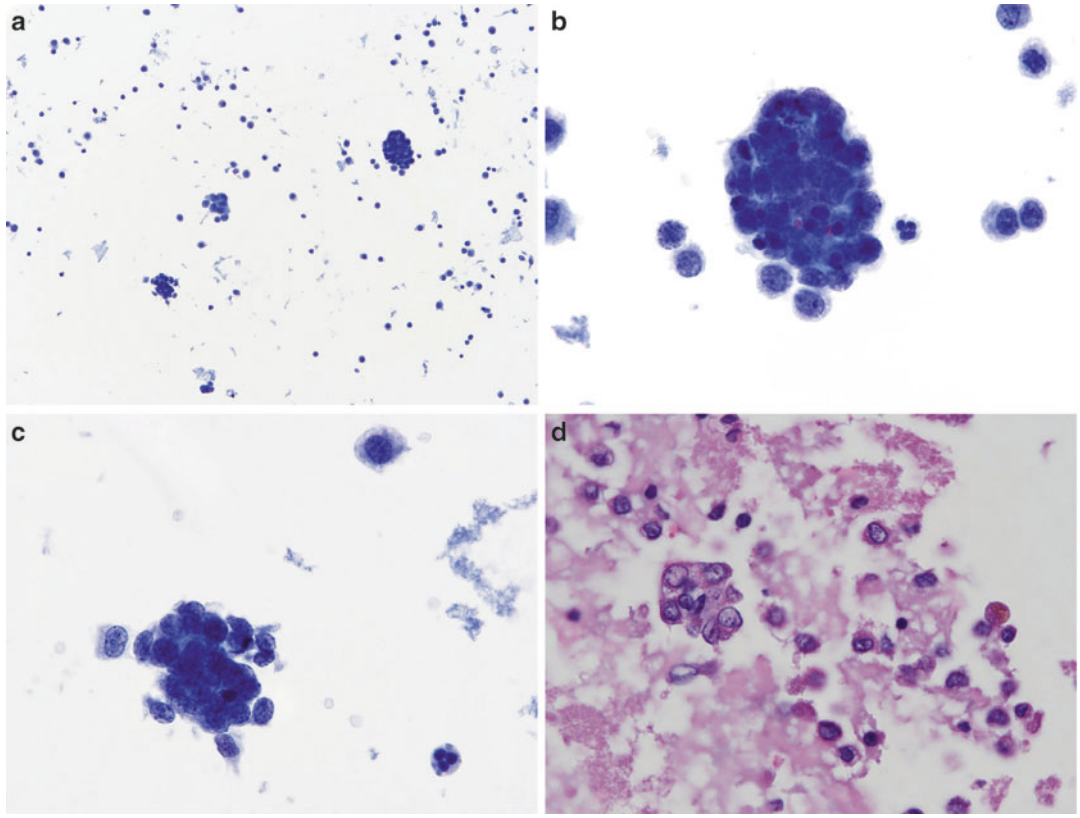
E

Microscopy

Cytology

Cells from endometriosis of cervix or vagina may be sampled during a Pap test. Endometriosis may be seen as spontaneously exfoliated endometrial cells which can present as three-dimensional tight clusters of small cells with high nuclear to cytoplasmic ratio. The degenerative nuclei are small (as big as intermediate squamous cell nuclei), uniform, round to oval with inconspicuous nucleoli and scant vacuolated cytoplasm. Directly sampled endometriosis is similar to directly sampled lower uterine segment and appears as two-dimensional sheets or tissue fragments with small uniform and polarized cells containing small tightly packed dark, round or oval nuclei with condensed chromatin and scant cytoplasm. Occasionally, peripheral nuclear palisading and tubular arrangements may be observed. Endometrial cells admixed with deep stromal cells and hemosiderin-laden macrophages suggest endometriosis.

Endometriosis can also be sampled and diagnosed by fine needle aspiration (FNA) and peritoneal washings when combined with clinical and surgical correlation. Presence of all three elements such as endometrial stromal cells, endometrial epithelial cells, and hemosiderin-laden macrophages is necessary for accurate diagnostic for endometriosis. Stromal cells have oval nuclei and extremely scant cytoplasm. Cell blocks can be useful if all three components necessary for a diagnosis of endometriosis are evident.



Endometriosis, Cytological Findings, Fig. 2 PW from a case of pelvic endometriosis in a 35-year-old patient who presented with bilateral ovarian cysts, histologically proven to be endometriomas (chocolate cysts). (**a**) PW shows dispersed small hyperchromatic groups (*HCG*) which on higher magnification were endometrial epithelial cells (2 o'clock position) and endometrial stromal cells (7 o'clock position) surrounded by macrophages, some contained pale golden hemosiderin (Pap stained TP, low magnification); (**b**) 3D cluster of endometrial epithelial cells. The degenerative nuclei are small (as big as a neutrophil, not at 3 o'clock position), uniform, oval with dark smudged chromatin, few nucleoli, and scant cytoplasm. Note surrounding macrophages with some containing pale golden hemosiderin granules; (**c**) A tight cluster of stromal cells with oval hyperchromatic nuclei and extremely scant or no visible cytoplasm (b and c, Pap stained TP, high magnification); (**d**) Cell block shows all three components necessary for diagnosis of endometriosis including endometrial epithelial and stromal cells and hemosiderin-laden macrophages (H&E stained CB, high magnification)

Histology

Histopathological features of lesions of endometriosis are similar to benign and normal endometrium. Immunohistochemistry has been found to be useful in diagnosing endometriosis as stromal cells are positive for surface antigen CD10 and epithelial cells are positive for cytokeratin.

Differential Diagnosis

Cytological differential diagnosis includes directly sampled endometrium and AIS. It may not be possible to distinguish endometriosis from the former based on morphology alone. Endometriosis can be distinguished from AIS by lack of nuclear atypia and small nuclear size. The presence of stroma and hemosiderin-laden macrophages favors endometriosis.

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Eosinophilic Effusions

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Synonyms

EPEs; EPFs; Pleural eosinophilia

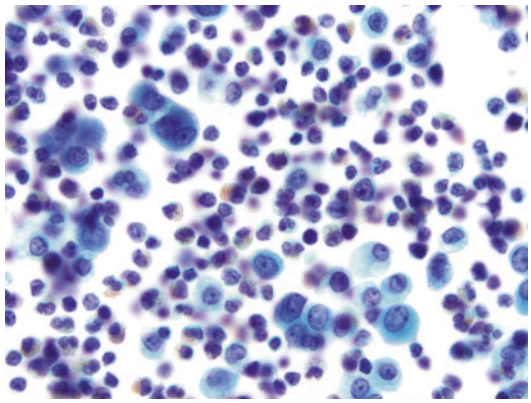
Definition

Eosinophilic effusions are defined as the accumulation of greater than 10% eosinophils by automated or manual differential in a body cavity, typically the pleural cavity (Hirsch et al. 1979). This type of effusion is relatively rare accounting for up to 16% of all exudative effusions (Kalomenidis, J & Light, R. W., 2003). Causes of eosinophilic effusions remain controversial. Older literature suggested an association with benign diseases; however, several recent prospective studies have not supported this association (Kalomenidis, J & Light, R. W., 2003; Özkara et al. 2007). Regardless of the stimulus, injury to the mesothelial lining results in recruitment of eosinophils from the bone marrow over a variable time course.

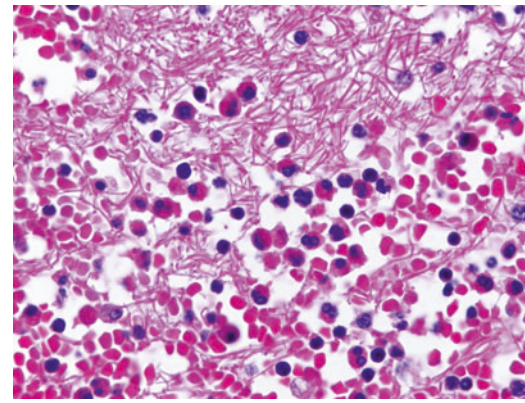
The most commonly referenced cause of pleural eosinophilia is spontaneous pneumothorax. In such cases, an extensive pleuritis and effusion occurs rapidly, often within just a few hours (Krenke et al. 2009). Hemothorax in the setting of repeated iatrogenic intervention (thoracentesis/thoracotomy) or trauma is probably the second most commonly cited cause. In contrast to pneumothorax, the effusion occurs over a much slower time course requiring several days to fully develop (Martínez, G, et al. 2003). Other frequent causes include malignancies of the lung and pleura and a variety of infections; in particular mycobacterial, fungal, and parasitic (reports include *Echinococcus, Wuchereria bancrofti, Schistosoma*, and *Strongyloides*). Other rare causes include asbestos, pulmonary embolus/infarction, and autoimmune and inflammatory conditions such as Churg-Strauss syndrome and rheumatoid pleuritis. Eosinophilic effusions, in some cases, may be the unintended side effect of certain medications; however, up to 25% of effusions are idiopathic: a diagnosis of exclusion (Oba, Y., & Abu-Salah, T., 2012).

Clinical Features

- **Incidence**
  Eosinophilic effusions are relatively rare and account for 5–16% of exudative effusions.
- **Age**
  May occur at any age; however, it was reported to occur mainly in male patients ranging between 22 and 95 years old with an average age of 67.4 years (Rubins, JB and Rubins HB, 1996).
- **Sex**
  Predominate in males with frequency of 63% males to 37% females (Özkara et al. 2007).



Eosinophilic Effusions, Fig. 1 High-power Papanicolaou stain demonstrates a reactive mesothelial population in a background of numerous leukocytes with bilobed nuclei and granular somewhat orangeophilic cytoplasm consistent with eosinophils

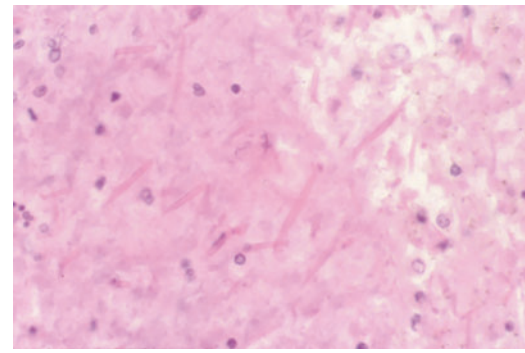


Eosinophilic Effusions, Fig. 2 Medium power H&E stain of cell-block demonstrates effusion with numerous eosinophils containing dense *red* cytoplasmic granules

- **Site**
  Eosinophilic effusions most often occur in the pleural cavities and are distinctly less common in the pericardium or peritoneum. In the pleura, it manifests as a right pleural effusion in 58.3%, left in 18.3%, and bilateral in 23.3% (Özkara et al. 2007).
- **Treatment**
  Thoracentesis and treatment of underlying etiology are usually sufficient. Most such lesions are due to an occult inflammatory process or medication effect and eventually resolve spontaneously without intervention. Treatment is based on the specific cause; however, idiopathic effusions should be followed until resolution or determination of the etiology.
- **Outcome**
  The prognosis of idiopathic effusions, even when they are persistent, is excellent. Effusion serologies, computed tomography, and pleural biopsy can sometimes aid diagnosis.

Macroscopy

Eosinophilic effusions are often viscous and can appear purulent.



Eosinophilic Effusions, Fig. 3 High-power Papanicolaou stain demonstrates an eosinophilic effusion with numerous Charcot-Leyden crystals. These *needle*-like shapes should not be confused with fungal hyphae

Microscopy

Liquid-based preparations are typically very cellular. The cytoplasmic granules on alcohol-fixed Papanicolaou-stained slides may be inconspicuous with a hint of orangeophilia (Fig. 1). The diagnosis hinges on recognition of a bilobed nucleus. Air-dried conventional Romanowsky-stained slides, however, demonstrate the characteristic bright eosinophilic cytoplasmic granules present in H&E-stained formalin-fixed tissue specimens (Fig. 2). Charcot-Leyden crystals (Fig. 3) are present in select cases and were reported to occur when the fluid had been refrigerated for at least 24 h (Naylor, B and Novak, PM, 1985).

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Ependymoma, Cytological Findings

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Definition

Neoplasm originating from ependymal cells lining the ventricles and spinal canal.

Clinical Features

- **Incidence**
  Ependymomas correspond to 2–9% of all neuroepithelial tumors. They are 6–12% of intracranial tumors in children. In the spine canal are the most common tumors corresponding to 50–60% of glial tumors.
- **Age**
  The ependymomas are predominantly tumors of infancy or childhood. Infratentorial ependymomas predominate in children with mean age of 6 years. They have a second peak at 30–40 years with lesion more frequently in spinal canal
- **Sex**
  The ependymomas have even distribution between men and women
- **Site**
  The lesions occur most frequently in fourth ventricle, arising from the floor or roof.
- **Treatment**
  The treatment of choice is surgery with excision of the lesion.
- **Outcome**
  The prognosis of these lesions is related to age, extent, and location of the lesion and histopathological grading. In children, the prognosis is worse due to frequent location in the posterior fossa in contrast to the spinal canal location in the adults. The survival in 5 years in children is around 50% and 57% in adults.

Macroscopy

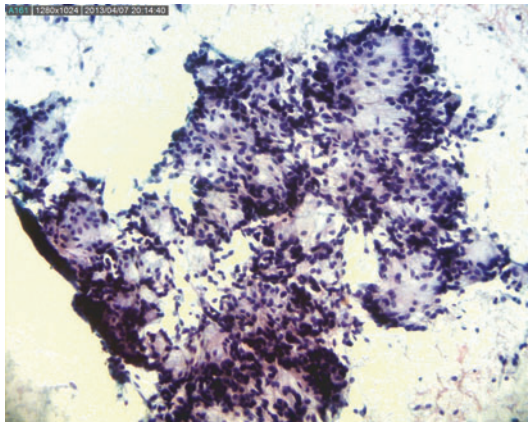
The lesions appear as tumors firm, lobulated red-gray color, and are sharply demarcated in relation to the adjacent neural tissue. Foci of necrosis or hemorragia are unusual.

Microscopy

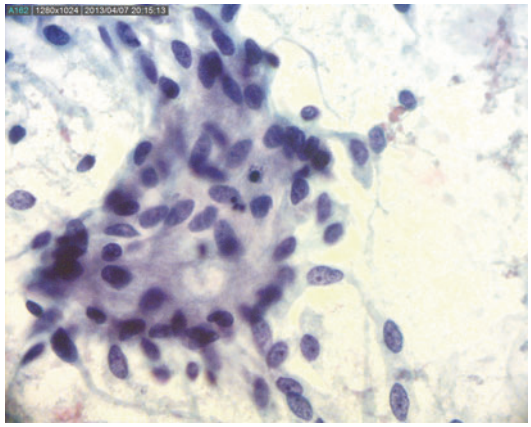
Ependymomas presents a moderately or high cellular tumor smeared in clumps with abundant matrix (Fig. 1), cells with a radiating, perivascular arrangement. The cells had fibrillary cytoplasm and elongated, sometimes carrot-shaped nuclei with delicate chromatin and regular nuclear membrane (Fig. 2). Very few mitosis or true ependymal pseudorosettes could be seen.

Immunophenotype

Most ependymomas express immunoreactivity for GFAP, S-100 protein, and vimentin. A high



Ependymoma, Cytological Findings, Fig. 1 Ependymoma – Note smear with high cellularity and abundant fibrillar matrix (Papanicolaou)



Ependymoma, Cytological Findings, Fig. 2 Ependymoma – Note cells with fibrillary cytoplasm and elongated, sometimes carrot-shaped nuclei with delicate chromatin, regular nuclear membrane (Papanicolaou)

percentage of ependymomas also demonstrate expression for EMA and variable number expressing focal positivity for cytokeratin.

Molecular Features

The most frequently genetic alteration is related to chromosome 22 in the forms of monosomy, deletions, or translocations. Supratentorial tumors preferentially showed loss of chromosome 9. The NF2 gene is involved in the genesis of these tumors.

Differential Diagnosis

The main differential diagnoses in ventricular lesions are choroid plexus tumors, intraventricular meningiomas, and metastases. The latter two are only likely in older age group and are usually easy to recognize in smear preparations. Choroid plexus tumors have epithelial features, cause more difficulty malignant lesions due the regular epithelial arrangement of tumors cells around blood vessels. In the spinal cord lesion, the most important differential diagnosis are astrocytomas. In astrocytic neoplasms the vascular papillary patterns are not associated with nuclear palisading and not observed relationship with blood vessels.

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Epithelial Proliferative Benign Lesion of the Breast, Cytological Findings

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Synonyms

Adenosis; Epithelial hyperplasia; Fibrocystic changes (FCC)

Definition

This terminology encompasses a group of breast lesions that includes sclerosing adenosis, collagenous spherulosis, usual and florid ductal epithelial hyperplasia, and columnar lesions without atypia. The cytological distinction between epithelial proliferative lesions with and without atypia is clinically useful, because patients with atypical lesions may be referred to surgical biopsy for a definitive diagnosis, while those with lesions without atypia may be managed more conservatively.

Clinical Features

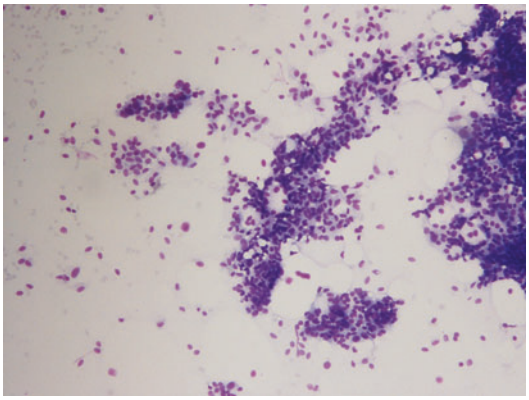
- **Incidence**
  More than one-third of women between the third and fifth decades of life show some evidence of fibrocystic changes.
- **Age**
  20–45 years old.
- **Sex**
  Female.
- **Site**
  Although FCC are frequently multifocal and bilateral, often the initial presentation is as a solitary lesion. The symptoms including premenstrual swelling, pain, and tenderness vary with the menstrual cycle. Clinical manifestations are more prominent during the reproductive cycle, and in the absence of hormonal replacement therapy, the symptoms of FCC generally cease in the first 2 years post menopause.
- **Treatment**
  Hormonal manipulation.

Macroscopy

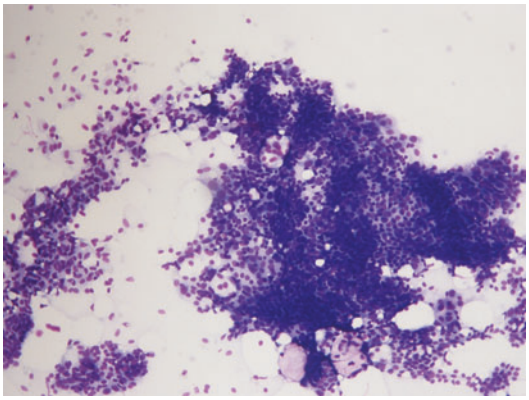
Variable aspects, in general areas of fibrosis with some cysts associated.

Microscopy

In these cases there is a mixture of features including cysts, fibrosis, and epithelial proliferation.

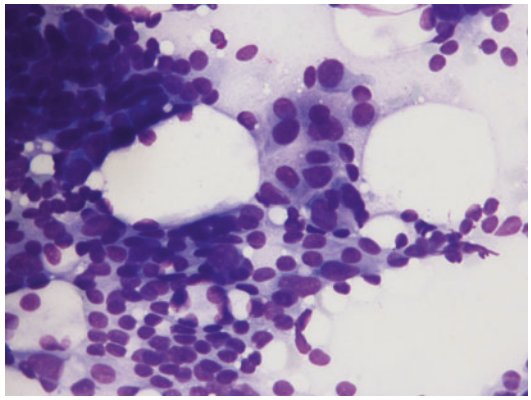


Epithelial Proliferative Benign Lesion of the Breast, Cytological Findings, Fig. 1 Benign epithelial proliferative lesion. Note the presence of numerous naked nuclei in the background of the smears (Giemsa stain)



Epithelial Proliferative Benign Lesion of the Breast, Cytological Findings, Fig. 2 Benign epithelial proliferative lesion. Monolayer sheets of regular ductal cells with presence of spindle dark cells intermingled (myoepithelial cells) (Giemsa stain)

Areas of proliferation are cellular, areas of fibrosis are rubbery and yield few cells, and cysts yield fluid. Small regular ductal cells are seen in monolayer sheets with apocrine cells and bare myoepithelial nuclei (Figs. 1–3). There may be thin proteinaceous fluid coating the slide. The nuclei of benign ductal cells have smooth nuclear membranes, fine and even chromatin, and inconspicuous nucleoli with 1.5–2 times bigger size than of a red blood cell. The hallmark of a benign FNA is the presence of bipolar myoepithelial nuclei.



Epithelial Proliferative Benign Lesion of the Breast, Cytological Findings, Fig. 3 Benign epithelial proliferative lesion. Observe together with the sheets of ductal cells, the presence of cells with large cytoplasm (apocrine metaplasia) (Giemsa stain)

Immunophenotype

Myoepithelial nuclei are oval, the chromatin is evenly distributed, the nuclear membrane is smooth, the nuclei are devoid of nucleoli, and these cells are strongly positive for p63. Other myoepithelial markers (smooth muscle actin, calponin, CD10, caldesmon, maspin) can also highlight the myoepithelial cells among the groups of epithelial cells.

Molecular Features

Most of the benign epithelial proliferative lesions of the breast do not have specific genetic alterations. The alterations related with the hyperplastic epithelial lesions such as atypical hyperplasia and columnar cell lesions are describe elsewhere in the relevant sections of the Encyclopedia.

Differential Diagnosis

Cytological diagnosis and classification of FCC with proliferative lesions can be very difficult because there is a significant overlap of cytological features among the different lesions, notably usual ductal hyperplasia, sclerosing and tubular adenosis, radical scar/complex sclerosing lesions, atypical hyperplasia, and even tumors such as papillomas and low-grade ductal carcinoma in situ. Thus, in cases where the cytological features are not classical of FCC (such as hypercellularity or some loss of cohesiveness of the cells and some degree of cellular atypia), the most important diagnostic decision is to define if the cytological pattern is benign, indeterminate, or malignant instead of trying to allocate the cytological preparation into a specific histologic diagnosis or pattern. In such situations, the definition of benign proliferative epithelial lesions (or proliferative FCC without atypia) and proliferative epithelial lesions (or proliferative FCC with atypia) may be useful and conveys the appropriate clinical significance. In the former situation, the recommendation is clinical and radiologic follow-up, but in the latter situation, further investigation is necessary, using, for example, core needle biopsy or surgical biopsy.

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Epithelial Proliferative Lesion of the Breast, Cytological Findings

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Synonyms

Atypical hyperplasia; Epithelial hyperplasia

Definition

The term "epithelial proliferative lesion of the breast" is used on cytology to describe a situation where it is not possible to define the precise nature of the epithelial proliferation. This terminology can encompass a group of breast lesions that includes sclerosing adenosis, collagenous spherulosis, usual and florid ductal epithelial hyperplasia, columnar lesions without and with atypia, atypical hyperplasia, and even low-grade DCIS. Patients with this cytological diagnosis must be referred to surgical biopsy for a definitive diagnosis.

Clinical Features

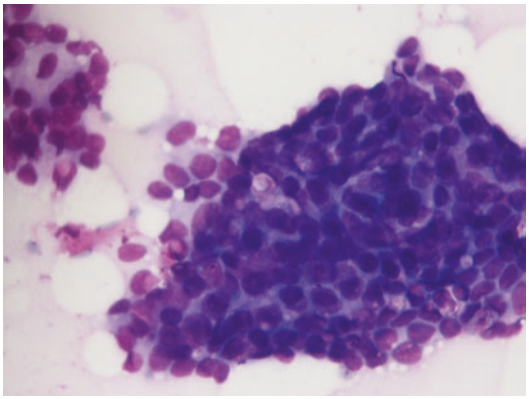
- **Incidence**
  Variable in different series. Around 20% will be malignant at histology.
- **Age**
  Variable.
- **Sex**
  Predominantly in women; however, some cases of gynecomastia and low-grade carcinomas of male breast can be diagnosed in cytology as epithelial proliferative lesions.
- **Site**
  Most of these cases are usually asymptomatic, only detected by the associated calcification at mammography or as incidental findings. There is no specific preferential site.
- **Treatment**
  The type of treatment depends of the definitive histological diagnosis.

Macroscopy

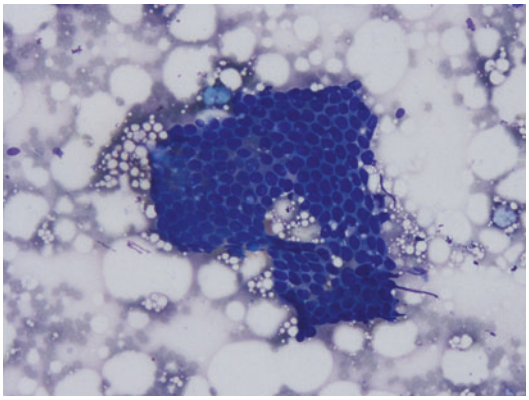
There are no reliable macroscopic features that are variable according to the correspondent histological diagnosis.

Microscopy

The cytological features for this category classically show most of the characteristics of a benign smear, but with some worrisome features

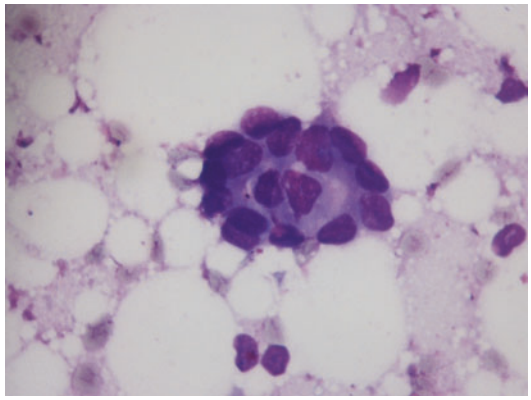


Epithelial Proliferative Lesion of the Breast, Cytological Findings, Fig. 1 Epithelial proliferative lesion. Group of cells showing cellular crowding (Giemsa stain)



Epithelial Proliferative Lesion of the Breast, Cytological Findings, Fig. 2 Epithelial proliferative lesion. Group of cells showing slight nuclei atypia, some crowding, forming regular spaces (Giemsa stain)

including cellular crowding, pleomorphism, and discohesion. The aspects of atypical duct hyperplasia and low-grade DCIS show variable cytology. The cells show atypical proliferative changes characterized by monotonous, evenly spaced epithelial cells showing round, slightly enlarged nuclei with fine chromatin and inconspicuous nucleoli (Figs. 1 and 2). Cellular cohesion may be reduced, resulting in variable to high number of single epithelial cells. The cell arrangement for the most part is cribriform, but solid and micropapillary patterns may be encountered. Rarely myoepithelial cells may be identified. In the background, a mixed proliferative epithelial cell population with or with atypia may be present.



Epithelial Proliferative Lesion of the Breast, Cytological Findings, Fig. 3 Epithelial proliferative lesion. Scant smear showing some tubules with nuclei pleomorphism. Observe some cell discohesion (Giemsa stain)

Associated necrosis with scattered foamy histiocytes and calcification may be present (Fig. 3).

Immunophenotype

Myoepithelial markers (including P63 and CK5) are negative in most of the groups. In cases of atypical hyperplasia and low-grade DCIS, there is a strong and homogeneous staining of the cells for estrogen receptor.

Molecular Features

The alterations related with the hyperplastic epithelial lesions such as atypical hyperplasia and columnar cell lesions and low-grade DCIS are describe elsewhere in the relevant sections of the Encyclopedia.

Differential Diagnosis

Cytological diagnosis and classification of FCC with proliferative lesions can be very difficult because there is a significant overlap of cytological features among the different lesions, notably usual ductal hyperplasia, sclerosing and tubular adenosis, radical scar/complex sclerosing lesions, atypical hyperplasia, and even tumors such as papillomas and low-grade ductal carcinoma in situ. Thus, in cases where the cytological features are not classical of FCC (such as hypercellularity or some loss of cohesiveness of the cells and some degree of cellular atypia), the most important diagnostic decision is to define if the cytological pattern is benign, indeterminate, or malignant instead of trying to allocate the cytological preparation into a specific histological diagnosis or pattern. In such situations, the definition of benign proliferative epithelial lesions (or proliferative FCC without atypia) and proliferative epithelial lesions (or proliferative FCC with atypia) may be useful and conveys the appropriate clinical significance. In the former situation, the recommendation is clinical and radiologic follow-up, but in the latter situation, further investigation is necessary, using, for example, core needle biopsy or surgical biopsy.

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Epithelial-Myoepithelial Carcinoma of the Salivary Gland, Cytological Findings

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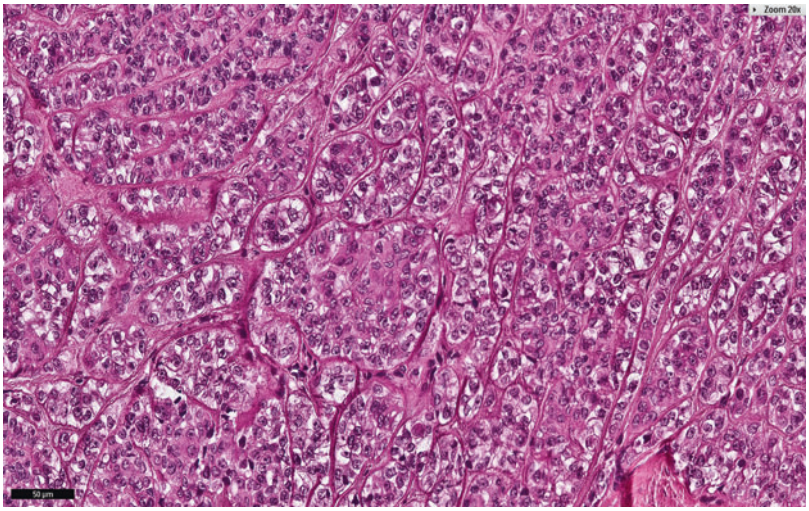
Synonyms

Clear cell carcinoma



Epithelial-Myoepithelial Carcinoma of the Salivary Gland, Cytological Findings, Fig. 1 Epithelial-myoepithelial carcinoma. Proliferation of clear cells and some darker, epithelial cells (H&E stain)

Definition

Epithelial-myoepithelial carcinoma (EMEC) is a low- or intermediate-grade salivary carcinoma (Barnes et al. 2005; Ellis and Auclair 2008) composed by a dual cell population of large, clarified, and external cells of myoepithelial lineage and inner, dark, and small ductal cells of epithelial origin (Fig. 1). A variant, when clear cells predominate and darker epithelial cells are inconspicuous or exclusive, is called clear cell carcinoma and represents a distinct entity. Clear cell carcinomas showing hyalinized stroma are called hyalinizing clear cell carcinoma.

Clinical Features

- **Incidence**
  EMEC accounts for less than 1% of salivary gland tumors.
- **Age**
  EMEC arises in all ages. The peak of incidence is between the sixth and seventh decades.
- **Sex**
  There is no sex predilection.
- **Site**
  Major and minor salivary glands are affected.
- **Treatment**
  Surgery is an initial treatment.
- **Outcome**
  Recurrences are rare. Metastases are unusual.

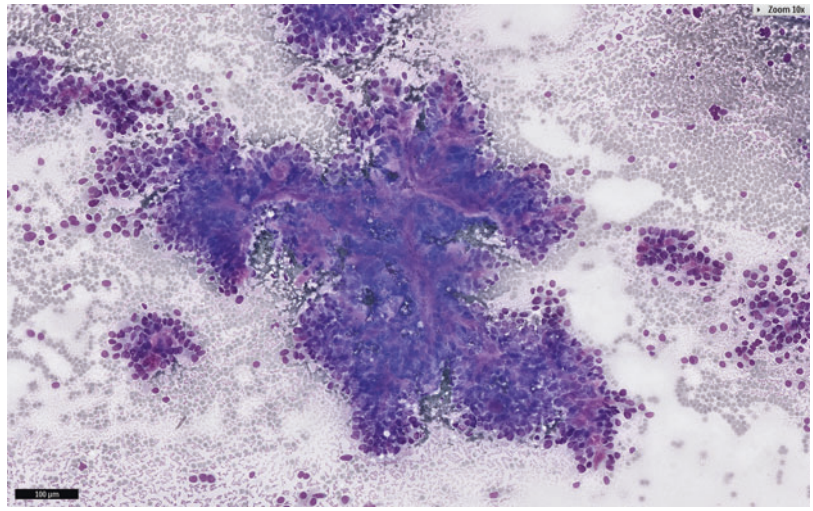
Macroscopy

EMEC is poorly circumscribed grayish mass. Necrotic and cystic areas may be seen.

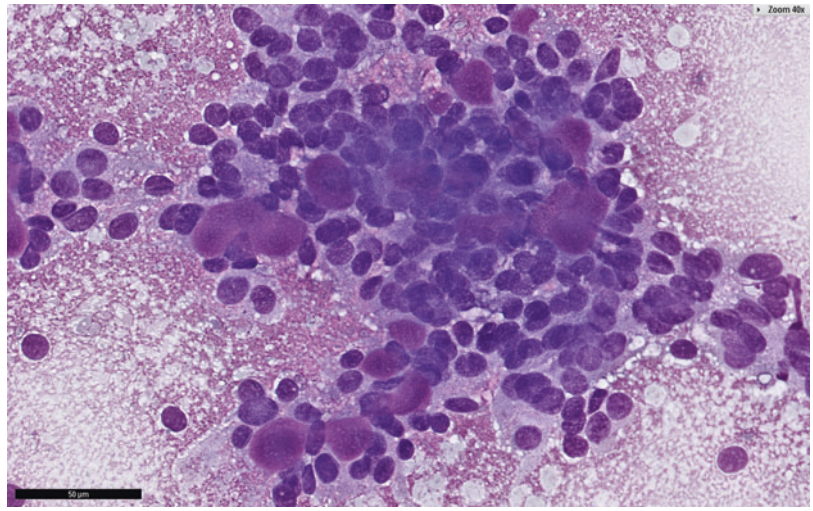
Microscopy

Cytology smears in epithelial-myoepithelial carcinoma (EMEC) are usually hypercellular and cell-rich and stroma-rich (Klijanienko and Vielh 1998a). EMEC belongs to the group of tumors exhibiting predominant myoepithelial cell morphology.

EMEC present a wide spectrum of cellular differentiation, and tumors may be exclusively composed of clear myoepithelial cells (clear cell carcinoma) or may contain a mixture of epithelial ductal cells distributed in round or cylindrical groups surrounded by large, clear myoepithelial cells. In smears, the cellular material always includes both isolated and clusters of oval cells. The cells are frequently arranged in pseudopapillary formations with intensely eosinophilic stromal



Epithelial-Myoepithelial Carcinoma of the Salivary Gland, Cytological Findings, Fig. 2 Epithelial-myoepithelial carcinoma. Tumor cells are frequently arranged in pseudopapillary formations with intensely eosinophilic stromal cores as tubular structures (MGG stain)



Epithelial-Myoepithelial Carcinoma of the Salivary Gland, Cytological Findings, Fig. 3 Epithelial-myoepithelial carcinoma. Hyaline globules (MGG stain)

cores (MGG stain) (Fig. 2). The cytoplasm is either abundant and slightly eosinophilic or scant. The cells rarely present a vacuolated cytoplasm, and, interestingly, no obvious clear cells are seen using MGG stain, but clear cells may be demonstrated using Papanicolaou stain. Plasmacytoid cells and naked nuclei are occasionally seen.

Stromal component consists in hyaline globules, similar to those of adenoid cystic carcinoma, tubular structures similar to those of pleomorphic low-grade adenocarcinoma (Fig. 3). Scant myxoid background and inflammatory cells may also be observed in the background (Klijanienko and Vielh 1998a).

Immunophenotype

Cytokeratins and EMA are strongly positive for epithelial cells. Clear cells react for muscle-specific actin, S-100, and vimentin. Myoepithelial markers in clear cell carcinomas are negative, and cytokeratins, S-100, EMA, or CEA are inconstantly

positive. Hyalinizing clear cell carcinoma (HCCC), an entity described in 1994 (Milchgrub et al. 1994), also lacks myoepithelial differentiation and expresses squamous differentiation (diffuse positivity with antibody ant-p63 and 34betaE12).

Molecular Features

Upregulation of the *WT-1* gene has been described. HCCC harbors a EWS1 rearrangement fusing the *EWS1* gene with *ATF1* (Weinreb 2013).

Differential Diagnosis

EMEC should be differentiated from other salivary tumors with myoepithelial cell predominance such as adenoid cystic carcinoma (ACC), polymorphous low-grade adenocarcinoma (PLGA), and cellular pleomorphic adenoma but also with tumors showing clear cells such acinic cell carcinoma (AcCC), HCCC, and metastatic renal cell carcinoma.

The differentiation between EMEC and ACC may be difficult due to the occasional presence of hyaline globules and three-dimensional cellular aggregates of basaloid cells which show clarified cytoplasms at the periphery of clusters. The presence of finger-like and tubular structures is strongly in favor of ACC (Klijanienko and Vielh 1997a). PLGA may be undistinguishable from EMEC due to the presence of pseudopapillary architecture, tubular structures, and hyaline globules. In PLGA cells are elongated and nuclei are clarified (Klijanienko and Vielh 1998b). However, PLGA arise frequently in the accessory salivary glands (palate), whereas EMEC arise usually in the parotid gland. Well-differentiated AcCC is characterized by clusters of cells with small nuclei and a finely vacuolated cytoplasm similar to normal acinic cells. In moderately differentiated cases, AcCCs are composed of less characteristic adenocarcinomatous cells and may be readily differentiated from EMEC. Moreover, hyaline globules and other stromal components are absent in AcCC (Klijanienko and Vielh 1997b). HCCC may be suggested by its immunoprofile and the EWS1 rearrangement detectable by FISH (Milchgrub et al. 1994; Weinreb 2013). Metastasis from a renal cell carcinoma should be always excluded.

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Epithelioid Sarcomas, Cytological Findings

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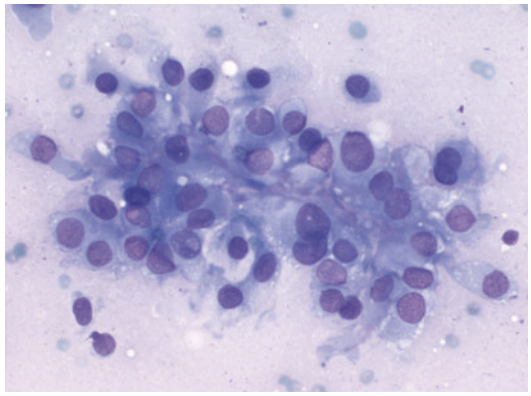
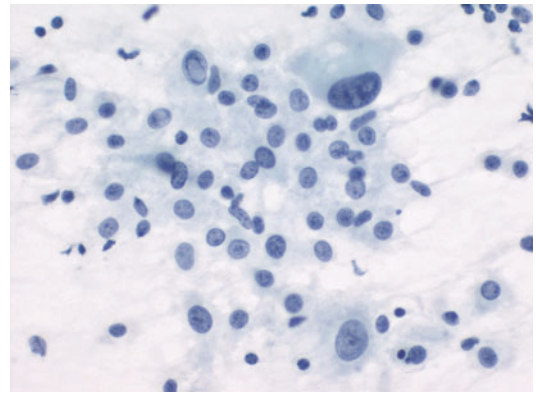
Definition

True epitheloid sarcomas are rare low-grade tumors with a slow relentless course. Epitheloid variants of angio-, rhabdomyosarcoma, malignant peripheral nerve sheet tumor (MPNST), and hemangioendothelioma are separate entities with different clinical presentation and behavior (Fig. 1).



Epithelioid Sarcomas, Cytological Findings, Fig. 1 Epitheloid sarcoma: monomorphic cohesive tumor cells with round nuclei and large fragile cytoplasm. MGG



Epithelioid Sarcomas, Cytological Findings, Fig. 2 Epitheloid sarcoma: loose cluster of tumor cells with round or oval nuclei with variation in size and indistinct cytoplasm. Intranuclear inclusion is observed. Papanicolaou

Clinical Features

- **Incidence**
  Rare, 0.1/1,000,000.
- **Age**
  Young adults between 10 and 39 years of age.
- **Sex**
  Male patients outnumber females, about 2:1.
- **Site**
  Distal parts of the extremities. Subcutaneous or deep soft tissue.
- **Treatment**
  Surgery, radiation therapy.
- **Outcome**
  Often follows a relentless course with local recurrences and distant metastasis.

Microscopy

FNA (fine needle aspiration) shows relatively uniform cells with round to oval nuclei with large fragile cytoplasm. Admixture of necrosis, collagen fragments, and inflammatory cells.

Immunophenotype

Vimentin, cytokeratin, CA125, and epithelial membrane antigen (EMA) positive. Some cases express CD34, S-100, and CD31 are consistently negative (Fig. 2).

Molecular Features

Loss of INI 1-gene in a majority of patients.

Differential Diagnosis

Squamous cell carcinoma, melanoma, and various epitheloid soft tissue tumors.

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Extrauterine Adenocarcinoma, Cytological Findings

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Synonyms

Extrauterine adenocarcinoma; Metastatic adenocarcinoma to lower female genital tract

Definition

Extrauterine adenocarcinoma involving the lower female genital tract (cervix or vagina) detected by cervicovaginal smear (Pap test) cytology. In the majority of the cases, the primary tumor is clinically known and cervical or vaginal involvement is a part of generalized metastatic spread.

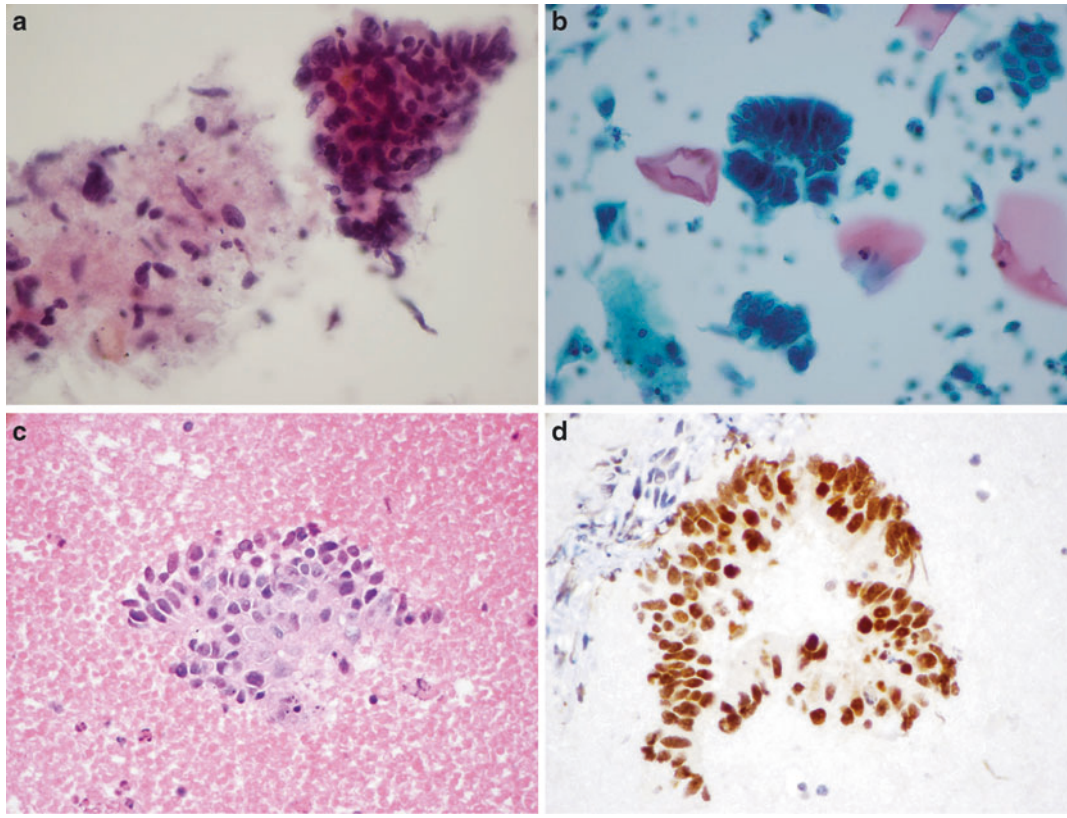
Clinical Features

- **Incidence**
  The lower female genital tract (cervix or vagina) is rarely involved by metastatic tumors. The reported incidence is approximately 0.004–5%. Most of the literature on cervicovaginal smear (Pap test) detection of extrauterine adenocarcinoma is in the form of individual case reports or brief accounts of a few cases. Metastasis from extrauterine adenocarcinoma is rarely a primary event and usually occurs in clinically known, widely disseminated, and advanced stage III or IV adenocarcinomas. Clinical history, correlation of Pap test cytology with histology of primary tumors, and immunocytochemistry are important for accurate detection (Fig. 1).
- **Site**
  Approximately 50% of tumors metastatic to cervical and vaginal tissue detected by Pap test cytology are of gynecological origin, arising from the ovary and fallopian tube, with the remaining being extrauterine. Overall, ovary, breast, and gastrointestinal tract are considered to be the most common extrauterine adenocarcinomas to metastasize to cervical and vaginal tissue and account for more than 2/3rd of the cases. Ovaries and vagina are the most frequent sites of metastasis, regardless of the location of the primary tumor and 81% of the cases involve one of these two sites. Metastasis to cervix is extremely rare. The latter may be explained due to the high fibromuscular tissue content of the cervix, which is believed to be an unfavorable medium for metastatic growth. The cervix also has a relatively limited blood supply, compared to that of the liver, lungs, brain, bones, or ovaries. Metastasis from extrauterine adenocarcinomas could result from direct extension from neighboring organs, such as colon, rectum and urinary bladder; via a patent fallopian tube from an ovarian, fallopian tube, or peritoneal primary; from distant sites via hematogenous dissemination; or retrograde lymphatic spread from breast, upper gastrointestinal tract, kidney, skin, and other distant primary sites. Metastatic involvement of the uterine cervix poses a diagnostic dilemma for both the clinician and the pathologist as metastasis, being so rare, may be overlooked.

Common Primary Sites to Metastasize to Lower Female Genital Tract

Ovary

Metastasis of ovarian adenocarcinoma to the cervix and vagina is very rare, with a reported incidence ranging from 3% to 5.3%. It is however the most common extrauterine metastasis to these sites. Usually, metastasis to the cervix involves

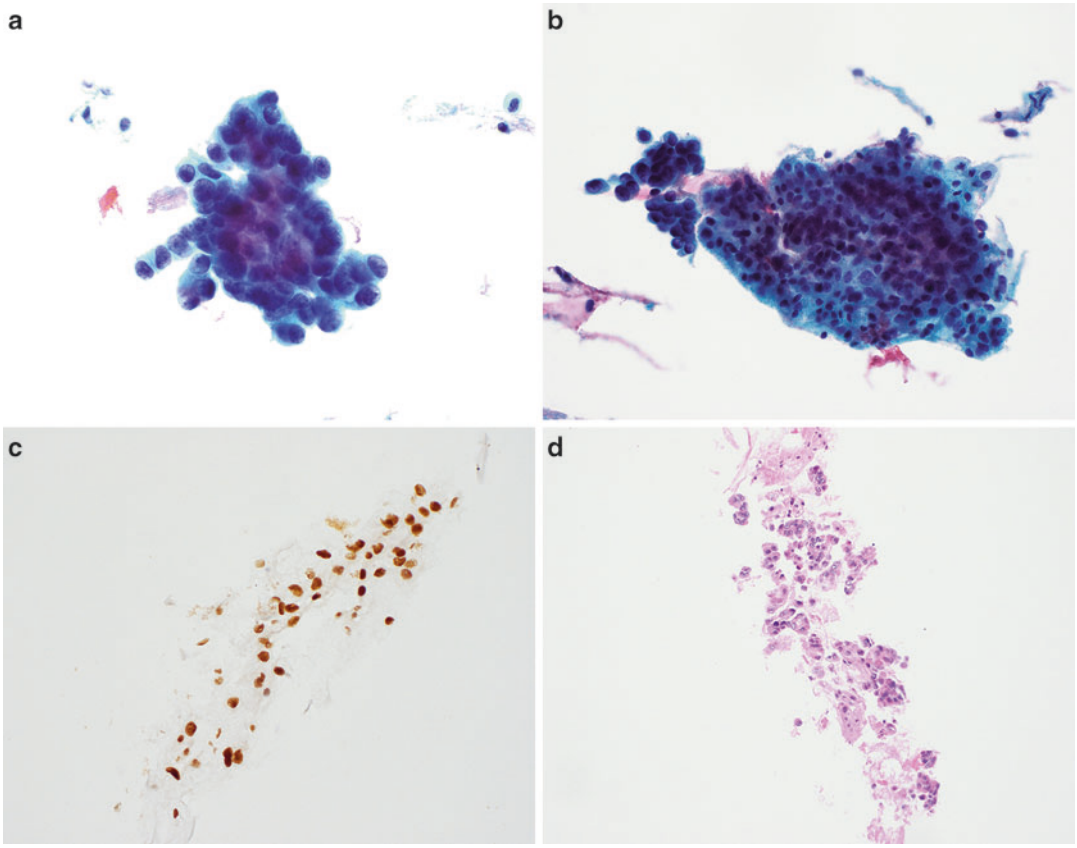


Extrauterine Adenocarcinoma, Cytological Findings, Fig. 1 (**a**) Metastatic colonic adenocarcinoma in a conventional Pap smear test. Smear shows "dirty" necrotic background diathesis with embedded single, elongated, "cigar-shaped" tumor cells. Note the hyperchromatic crowded group of tumor cells with nuclear stratification and peripheral palisading. Differential diagnosis includes primary cervical adenocarcinoma which may be distinguished from metastatic colonic adenocarcinoma by review of clinical history and pertinent histology. If a cell block is processed, immunostaining for colonic markers such as CDX-2 and endocervical markers, CEA and p16, can be performed (Pap-stained conventional smear, high magnification); (**b**) Metastatic colonic adenocarcinoma in a Pap test processed as SurePath. Although the cells appear in different planes of focus, the morphology is well maintained. Background necrosis is preserved, dispersed diffusely, and shows embedded elongated tumor cells. The groups show well-formed glands and strips of tumor cells with stratified nuclei that are elongated "cigar-shaped" (Pap-stained SurePath, high magnification); (**c**) Residual material from the latter Pap test was processed as a cell block which shows similar morphology as the Pap test (H&E stained cell block, medium magnification); (**d**) Immunostain for CDX-2 was positive (cell block section, medium magnification)

only vascular/lymphatic spaces and/or the deep cervical stroma; however, in exceedingly rare cases it may involve the surface epithelium and superficial cervical stroma which can be challenging to distinguish from primary cervical adenocarcinoma.

Breast

Breast carcinoma is also considered a common extrauterine malignancy to metastasize to the lower female genital tract (cervix and vagina). Metastasis is usually hematogenous from an advanced-stage widely disseminated disease but may rarely be an isolated event. Although, lobular carcinoma of the breast accounts for only 10% of breast malignancies in the United States, it is the most frequent breast carcinoma to metastasize to the lower genital tract. Lobular carcinomas are more likely to metastasize to the serosae, meninges, digestive, and genital tract than ductal carcinomas, which usually metastasize to lymph nodes, lungs, and brain. The mean interval for



Extrauterine Adenocarcinoma, Cytological Findings, Fig. 2 (**a**): Metastatic lobular carcinoma of breast in a Pap test processed as ThinPrep (*TP*). Tumor cells appear in a 3-D group. Background is clean and shows rare small squamous cells. The tumor cell clusters are round, enlarged (compare with the "bare" intermediate cell nucleus at 10 o'clock position), with round hyperchromatic and slightly irregular nuclei containing nucleoli, cytoplasm appears dense to finely vacuolated with occasional distinct vacuole (5 o'clock position). Note the linear arrangement of cells at the periphery of the group. This morphology is diagnostic of lobular carcinoma. Patient was 68-years old and had histologically proven lobular carcinoma diagnosed 5 years ago (Pap-stained TP, high magnification); (**b**) Note atrophic cervicovaginal cells closely associated with lobular carcinoma cells (9 o'clock position) (Pap-stained TP, medium magnification); (**c**) The residual material, left over after the TPPT, was processed, as a cell block. Note similar morphology as the TPPT (H&E stained cell block, medium magnification); (**d**) Immunostain for GATA-3 was positive and HPV test was negative (Cell block section, medium magnification)

detection of metastases is 40 months (range, 0–192; median, 28) from the diagnosis of primary adenocarcinoma. Diagnosis of lobular carcinoma in the Pap test may rarely be the primary manifestation of disease, if the tumor in the breast escapes clinical detection (Fig. 2).

Gastrointestinal Tract

A few cases of gastrointestinal adenocarcinoma, mostly with signet ring cell morphology and initially diagnosed by cervical cytology, have been reported to date. The primary tumors were of gastric, colorectal, pancreatic, and gallbladder origin.

- **Age**
  Varies with the type of primary malignancy and can be wide ranging.
- **Symptoms**
  Clinically, the patients usually present with abnormal vaginal bleeding or spotting, pain, and dyspareunia. In symptomatic patients, the

tumor is usually detected in a Pap test. Rarely, it is detected in Pap tests when symptoms are not attributable to genital tract such as bone pain or pathological fractures. Rarely, a cervical or vaginal tumor may be the presenting manifestation of a nongynecologic carcinoma.

- **Treatment**
  An accurate diagnosis of extrauterine adenocarcinoma in Pap test requires clinical information and corroboration by histological and immunohistochemical analysis. Extrauterine cancers can frequently masquerade as primary uterine neoplasms and present a cytologic dilemma in the differentiation of primary versus secondary adenocarcinoma. The distinction between primary and nonprimary cervical adenocarcinomas is critical, as it has clinical management ramifications with different prognosis and treatment. The usual treatment of metastatic extrauterine adenocarcinoma detected in Pap test is chemo- or radiation-therapy or total hysterectomy.
- **Outcome**
  Extrauterine adenocarcinomas that metastasize to the lower genital tract (cervix and vagina) are usually clinically widely disseminated advanced stage III or IV tumors and thus have poor prognosis.

Macroscopy

Vaginal examination typically reveals an eroded cervix. On colposcopy, an ectropion and an increased vascularity with irregularly distorted vessels may be visible. The cervix may show a polypoid endocervical mass. Rarely, the lesion may be located high up in the endocervical canal, making it difficult to visualize.

Microscopy

Histologic features that suggest the possibility of a metastatic origin for a cervical neoplasm include tumor location, histologic growth pattern, and lymphovascular invasion (LVI). Tumor is located predominantly in the outer aspect of the cervical wall, within the stroma and without involvement of the mucosal surface. Histologically, a multinodular or infiltrative growth pattern with entrapment of normal endocervical glands may be seen. Disease may be multifocal and show prominent vascular/lymphatic invasion. Dirty necrosis and diathesis is common when direct invasion occurs and is rare with peritoneal implants. Histologically, the usual type of primary endocervical adenocarcinoma, which comprises 80% of cervical adenocarcinomas, include: cells with eosinophilic or amphophilic mucin-poor cytoplasm, brisk mitotic activity, and frequent apoptotic bodies. In addition, moderately differentiated adenocarcinomas can have villous papillae that are located either on the tumor surface or within the glandular lumens. Primary endocervical adenocarcinomas need to be distinguished from the various types of metastatic extrauterine adenocarcinomas. The presence of a papillary serous pattern, a prominent signet-ring cell component, or a single file or dyscohesive arrangement of the tumor cells suggests the possibility of an ovarian, gastrointestinal, or breast origin, respectively. Review of clinical history, comparative histological analysis of the primary tumor, and immunohistochemical staining may be useful for distinguishing primary cervical carcinomas from metastatic carcinomas. Cytokeratin (CK) 7, CEA, and p16 are usually expressed in primary cervical adenocarcinomas.

Ovary

Typically, patients with ovarian carcinoma metastatic to the cervix and vagina have extensive peritoneal disease that can be associated with ascites and lymph node involvement. High-grade serous carcinoma of the ovary represents the most common type of carcinoma metastatic to the lower genital tract. The tumor is comprised of mildly to moderately atypical eosinophilic or amphophilic columnar cells arranged in glands and papillae. Apoptotic bodies are not seen. Psammoma bodies and intraglandular mucinous material are usually noted. Occasionally psamomma bodies are seen with benign

conditions, such as mesothelial hyperplasia, so it is important to ensure that associated cells show malignant features. Lymphovascular invasion may be extensive. There is no involvement of native mucosal surface (in-situ carcinoma). Cytologically, the cells may be degenerated. Background is usually clean (no tumor diathesis) and cells are frankly malignant in appearance with highly atypical nuclei consistent with serous type.

Lobular Carcinoma of Breast

Based on published literature, lobular carcinoma seems to metastasize to the cervix more frequently than ductal tumors which supports the assumption of a hematogenous spread.

Histological evaluation shows nests of tumor cells within the stroma. The tumor cells are small and arranged in single file pattern (linear arrangement of cells). Immunohistochemically, the tumor is typically positive for ER, PR, and GATA3. Cytologically, the signet ring cell morphology may be prominent in metastatic lobular carcinoma even when the primary tumor lacks signet ring cells. Three morphologic types of signet ring cells have been found in lobular carcinomas: the intracytoplasmic lumina type, with well-defined intracytoplasmic lumina, frequently containing eosinophilic material; the eosinophilic type, with pale, eosinophilic cytoplasm; and the goblet cell type, with abundant finely vacuolated cytoplasm. Cells are usually dispersed singly or in a linear arrangement (Indian-file pattern) or in small clusters.

Gastrointestinal

Cytology of metastatic adenocarcinoma in the Pap test may differ with direct extension versus spontaneous exfoliation from a peritoneal implant of the tumor. Colorectal adenocarcinoma is most likely to metastasize to vaginal wall via direct extension with the formation of rectovaginal fistula. Pap test shows a moderately cellular specimen with clusters, groups, and syncytial fragments of columnar-shaped tumor cells with enlarged ovoid nuclei, increased nuclear to cytoplasmic ratio, marked nuclear pleomorphism, hyperchromasia, and nucleoli in some of the cells. Vegetable material, mixed inflammatory cells, and necrotic diathesis are a characteristic background. When tumor cells spontaneously exfoliate in a Pap test from a peritoneal implant, the cell size may be smaller, cells are more closely packed in smaller groups with smooth contour, and the background is relatively clean. Immunohistochemically, tumor cells are positive for CK20 and CDX-2. Signet ring cell carcinoma shows large cells with a large mucin vacuole occupying the entire cytoplasm and displacing the nucleus to the periphery. Signet ring cell carcinoma is most commonly encountered in a Pap test from a gastric primary.

Useful Features to Suggest or Confirm but Are Not Specific of the Primary Origin Include

Papillary groups	Ovary, fallopian, peritoneal, kidney
Psammoma bodies	Ovary, fallopian, peritoneal
Single file	Lobular carcinoma of breast
Cigar-shape cells, dirty background	Colon
Signet ring cells	Gastric
Clear cytoplasm	Renal, ovary

Immunocytochemistry Cell blocks can be prepared from residual liquid-based cytology specimens to assess architecture and for immunohistochemical analyses (please see specific sections under "Microscopy").

Differential Diagnosis

In patients with atypical presentations of cervical adenocarcinoma, it is important to consider a metastatic tumor in the differential diagnosis. A thorough work-up to distinguish primary endocervical adenocarcinoma from metastatic disease is important before initiating therapy. Extrauterine metastases from ovary, breast, and gastrointestinal tract are the most common types. Review of clinical history, comparative analysis of histological material from the primary tumor, and an appropriate panel of immunohistochemical stains are important in the differential diagnosis.

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Eyelids Cytology, General Aspects

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Description

Skin lines the eyelids externally and the conjunctiva lines the inner surface; cytological sampling and microscopic examination of these areas are similar to those of other sites. Scraping is the most commonly used technique for cytological diagnosis of the skin and mucosa and may be performed by skillful cytopathologists, whereas sampling of lesions of the cantus or the rim should be performed by ophthalmologists. Scrapings may be performed without anesthesia using a specific spatula or the blunt side of a scalpel blade. When suspected lesions are not ulcerated, it may be necessary to lift or remove the external epidermal layer covering the lesion. This can be achieved by using the sharpened edge of a scalpel blade to gently cut through the superficial layer of the epidermis and thus allow the scraping of the deeper one. Smears are prepared as previously described and may be fixed in alcohol and stained by the Papanicolaou method or air-dried and stained with May-Grümwald-Giemsa (MGG) or Diff-Quik. As in other regions, MGG or Diff-Quik stainings are generally suitable for the demonstration of bacteria and to diagnose lymphoid and mesenchymal tumors, while the Papanicolaou method is preferable for epithelial or melanocytic tumors.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings

- Cornea Cytology
- Cytology of the Orbit and Ocular Adnexa
- Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- Meningioma, Cytological Findings
- Orbit Cytology, General Aspects
- Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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F

Fat Necrosis, Cytological Findings

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Synonyms

Steatonecrosis

Definition

Fat necrosis is a benign condition that consists of necrosis of breast fat tissue that has been bruised, injured, or has died. Fat necrosis can occur after any type of traumatic breast injury or surgery. A breast biopsy can also cause breast fat necrosis. Fat necrosis may also form around substances that have been injected into the breast, such as silicone or paraffin. Breast radiation treatment may sometimes cause an area of fat necrosis.

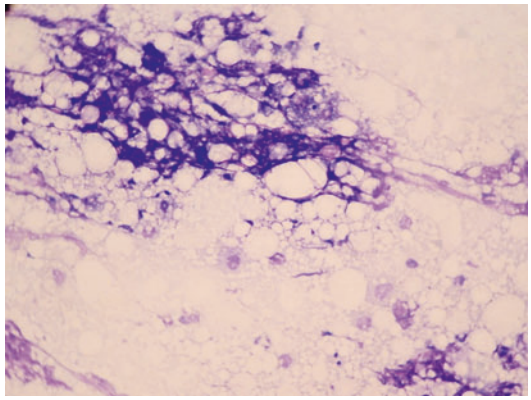
Clinical Features

- **Incidence**
  There is usually a history of injury 1 or 2 weeks before the lump is noted. But it may also follow surgery or radiotherapy.
- **Age**
  It may be encountered at any age.
- **Sex**
  Much more frequent in females but cases have been reported in man.
- **Site**
  Occur in any site and may present as a firm, fixed and painful mass. Sometimes there is skin retraction, including nipple retraction. Because of calcifications secondary to necrosis, the lesion can mimic malignancy on mammography.
- **Treatment/Outcome**
  It is variable. In some cases there are only need to medical treatment to relieve the symptoms because the lesion regress. Sometimes is need surgical excision of the nodule.

Macroscopy

Grossly fat necrosis is evident as a lump, which may or may not be bruising. It may be suspicious even with a hint of skin tethering in healing cases.

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4



Fat Necrosis, Cytological Findings, Fig. 1 Fat necrosis. Observe foam macrophages and necrotic fat tissue

Microscopy

The cytological aspirates tend to be thick, granular, and fatty when spread and contain foamy macrophages and variable number of multinucleate giant cells (Fig. 1). Other inflammatory cells may be present with usually a paucity of epithelial cells. The background contains fatty globules and fragments of adipose tissue. Sometimes atypical cells can be present, especially in cases secondary to radiation therapy.

Immunophenotype

No specific immunophenotype features have been reported.

Molecular Features

No specific molecular features have been reported.

Differential Diagnosis

Inflammatory lesions such as tuberculosis, granulomatous mastitis, or other causes of panniculitides may be mistaken for fat necrosis. Clinically it can imitate carcinoma closely. Failure to examine the active macrophages in a cellular aspirate from fat necrosis closely at high power can reinforce a wrong assumption by the inexperienced cytopathologist.

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Ferruginous Bodies

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Synonyms

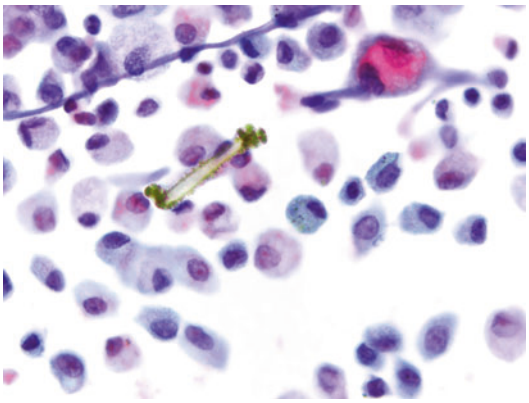
Asbestos bodies; Asbestosis bodies; Non-asbestos bodies; Pseudoasbestos bodies

Definition

Asbestos bodies were first described in 1906 by Marchland and were originally called peculiar "pigmented crystals." Later, the relationship between asbestos exposure and the peculiar crystals in the lung was identified, and in 1929, they were named "asbestosis bodies" which was later shortened to "asbestos bodies." In the 1960s, studies revealed that crystal structures resembling asbestos bodies could also be found with inhalation of numerous other types of fibrous dust including: silicates, carbon, talc, fiber glass, and other minerals. As a result, the term "ferruginous bodies" was created and is to be used when the

exact origin of the fiber is not known (Gross et al. 1968; Roggli et al. 1992). Generally, a ferruginous body is a term that refers to a mineral particle that has an iron coating deposited by the pulmonary macrophages. The vast majority of ferruginous bodies are asbestos bodies; however, the origin of the particle cannot definitively be identified by light microscopy alone.

Ferruginous bodies form following the inhalation of fiber particles into the distal alveolar spaces of the lung. Alveolar macrophages phagocytose the particle and cover it with a layer of iron-protein-mucopolysaccharide material, giving it a characteristic yellow-brown color.



Ferruginous Bodies, Fig. 1 High power Papanicolaou stain demonstrating a ferruginous non-asbestos body appearing as a *brown* straight fiber with dumbbell shape, yet a wide *yellow* central core inconsistent with asbestos bodies

Clinical Features

Not applicable

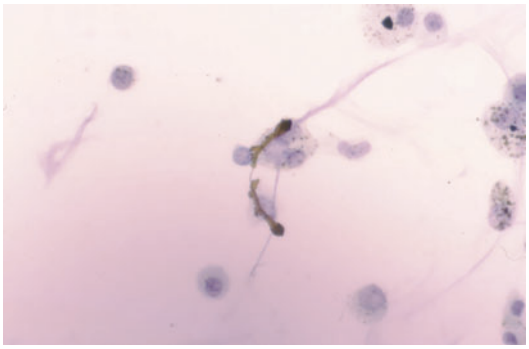
Macroscopy

Not applicable

Microscopy

Ferruginous bodies are typically golden brown in color due to the iron component of the coating. Often, they will appear as long rods which are beaded, segmented, or dumbbell shaped (Fig. 1). Occasionally, branched (resulting from iron deposition on a splayed fiber), curved, and circular forms can be seen. Ferruginous bodies are usually between 20 μm and 50 μm in length, with some reported up to 500 μm. Asbestos bodies are 2–5 μ in diameter and have a well-defined central smooth core along the length of the entire fiber (Fig. 2). Often they are identified within or surrounded by macrophages. The number of ferruginous bodies correlates with the duration and extent of exposure in most cases (Demay 2012; Roggli et al. 1992). Bronchoalveolar lavage is more sensitive for the identification of ferruginous bodies than is a sputum.

Ferruginous bodies can also be identified in pleural effusions. Pleural effusion is the most common presentation of asbestosis in the first 20 years after exposure. In addition to ferruginous bodies, an asbestos effusion may also contain eosinophils and multinucleated giant cells (Demay 2012).



Ferruginous Bodies, Fig. 2 High power Papanicolaou stain demonstrating a ferruginous asbestos body appearing as a *dark brown* straight beaded fiber with *dumbbell shape* and a well-defined straight central core

Special Stains

Iron stains including colloidal iron and Prussian blue will have a strongly positive reaction with ferruginous bodies due to the iron coating.

Differential Diagnosis

Ferruginous bodies have a characteristic appearance and usually are readily identifiable by light microscopy; however, the fiber of origin cannot be determined by light microscopy alone and requires additional studies such as electron microscopy for confirmative differentiation. The main diagnostic consideration which needs to be differentiated from ferruginous bodies is fungal organism which will be positive on a gomori methenamine silver stain and negative on an iron stain (Demay 2012; Roggli et al. 1992).

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Fibroadenoma, Cytological Findings

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Synonyms

Fibroepithelial benign tumor

Definition

Fibroadenoma is a breast benign neoplasm arising from the terminal-duct lobular unit (TDLU) and featuring a proliferation of both epithelial and stromal elements.

Clinical Features

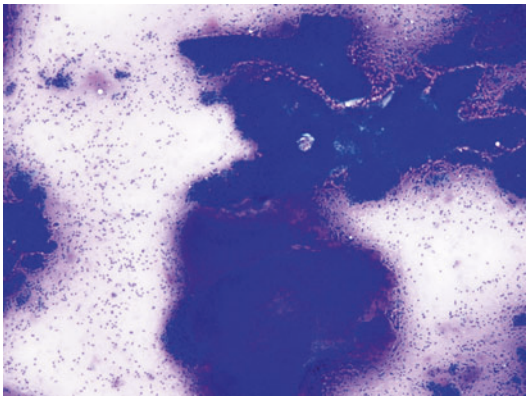
- **Incidence**
  Most frequently benign breast tumor.
- **Age**
  Women of childbearing age, especially those aged <30 years, although it may be encountered at any age.
- **Sex**
  The higher evidence is in female patients.
- **Site**
  There is not a specific site location. Fibroadenomas typically present as a painless solitary, firm, slow-growing, mobile, well-defined nodule of up to 3 cm in diameter. Less frequently it may occur as multiple nodules arising synchronously or metachronously in the same or in both breasts and may grow very large.
- **Treatment/Outcome**
  The majority of fibroadenomas do not recur after complete surgical excision. In adolescence there is a tendency for one or more new lesions to develop at another site or even close to the site of the previous surgical treatment.

Macroscopy

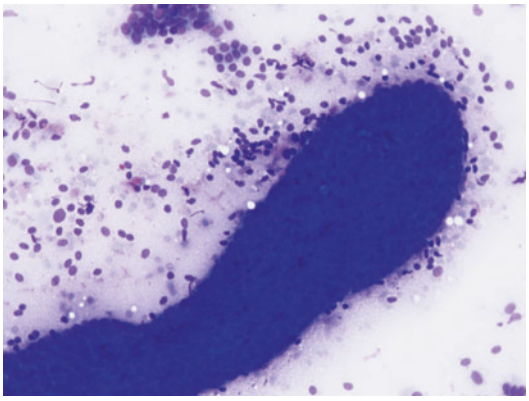
Grossly fibroadenomas are ovoid and well circumscribed. It shows a grey-white cut surface, solid, rubbery, bulging, with a slightly lobulated pattern and slit-like spaces. Hyalinization, myxoid changes, and calcification can be present.

Microscopy

The cytological diagnosis of fibroadenomas is often obvious at low power with characteristic

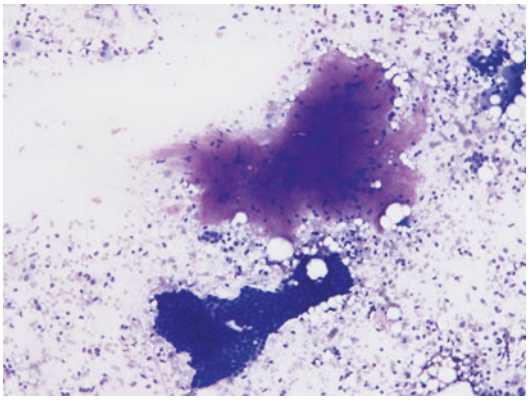


Fibroadenoma, Cytological Findings, Fig. 1 Fibroadenoma. Note the three main components in cytology: epithelial cohesive groups, stroma, and naked nuclei (Giemsa staining)



Fibroadenoma, Cytological Findings, Fig. 2 Fibroadenoma. "Fingerlike" projection in epithelial component of fibroadenoma (Giemsa staining)

large frond-like epithelial groups with peripheral fingerlike projections. At high power this epithelium is composed of closely packed, uniform cells with an irregular honeycomb appearance. The nuclei are approximately the size of one or two erythrocytes and are round or slightly ovoid, having one or two small nucleoli and finely granular chromatin. Myoepithelial cells are seen scattered over the surface of the sheets of ductal epithelial cells. The other essential feature is the presence of abundant naked bipolar cell nuclei which have condensed chromatin packed into a small elongated nucleus. In some fibroadenomas a scattering of foamy macrophages or apocrine cells is seen. Atypical epithelial cells can be found in some cases of fibroadenomas. Generally, the number of these cells is small and the presence of benign cytological findings (benign epithelial cells, myoepithelial cells, naked bipolar nuclei) helps to interpret the smears as benign (Figs. 1–3).



Fibroadenoma, Cytological Findings, Fig. 3 Fibroadenoma. Note the low cellularity in the stroma component (Giemsa staining)

Immunophenotype

There are no specific markers used, but P63 can be very useful to detect the nuclei of myoepithelial cells in doubtful cases. The naked nuclei in the smears of fibroadenoma are strongly positive for P63.

Molecular Features

Numerical abnormalities of chromosomes 16, 18, and 21 with one case of deletion of 17p have been reported in fibroadenomas. Clonality studies of fibroadenomas found predominant polyclonality of both epithelium and stroma, although monoclonality was observed in areas of stromal expansion, suggesting stromal progression. The DNA of fibroadenomas is less frequently methylated than that of phyllodes tumors.

Differential Diagnosis

The main differential diagnosis is phyllodes tumor. The stromal cells of phyllodes tumors exhibit elongated nuclei and delicate cytoplasm, resembling fibroblasts. In the smears of a fibroadenoma, stromal cells appear as small spindle cells immersed in fragments of fibromyxoid stroma and dispersed as bipolar naked nuclei. Atypical epithelial cells can be found in some cases of fibroadenomas. Generally, the number of these cells is small and the presence of benign cytological findings (benign epithelial cells, myoepithelial cells, naked bipolar nuclei) helps to interpret the smears as benign.

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Fibromatosis, Cytological Findings

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Synonyms

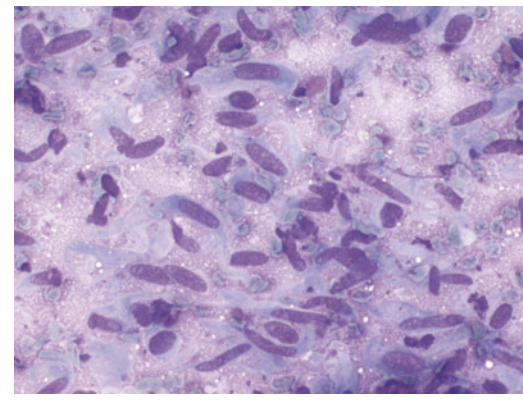
Aggressive fibromatosis; Deep fibromatosis (abdominal and extra-abdominal); Desmoids; Superficial fibromatosis (palmar, plantar, and penile fibromatosis)

Definition

1. Superficial fibromatoses are small benign fibroblastic proliferations which untreated may result in contractions.
2. Deep fibromatoses are slowly growing locally aggressive tumors.

Clinical Features

- **Incidence**
  1. Superficial fibromatosis, relatively common.
  2. Deep fibromatosis, 0.3/100.000.
- **Age**
  1. Superficial fibromatosis, above 30 years.
  2. Deep fibromatosis, young adults to middle aged.
- **Sex**
  1. Superficial fibromatosis, men dominate.
  2. Deep fibromatosis, women dominate the abdominal wall cases. Extra-abdominal tumors are equally distributed.
- **Site**
  1. Superficial (fascial) fibromatoses can be palmar (Dupuytren's disease), plantar (Ledderhose's disease), penile (Peyronie's disease), or over the extensor surfaces of the interphalangeal joints (knuckle pads) (Fig. 1).



Fibromatosis, Cytological Findings, Fig. 1 Plantar fibromatosis: spindle cells with elongated "*cigar-shaped*" nuclei with elongated distinct cytoplasm. MGG

2. Deep (musculoaponeurotic) fibromatoses can be abdominal, extra-abdominal (affect the muscles of the shoulder, pelvic girdle, and thigh), or intra-abdominal (pelvic, mesenteric).

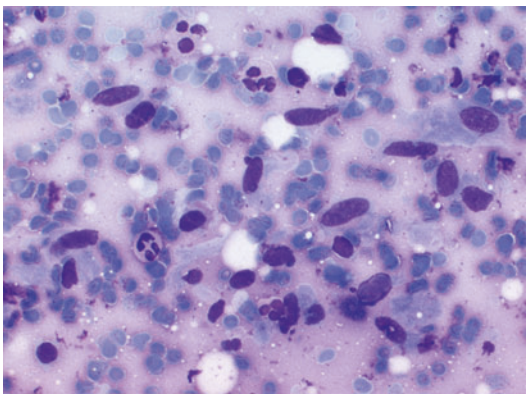
- **Treatment**
  Surgery.
- **Outcome**
  1. Superficial variants seldom recur after surgery.
  2. Deep fibromatosis recurrences are common and fatal outcome are not uncommon.

Microscopy

Fine needle aspiration cytology usually shows low to moderate cellularity of yield material composed of small fragments of fibrous stroma and collagen fibers admixtured with bland spindle-shaped cells with bipolar nuclear and elongated or oval naked nuclei. Large, multinucleate cells representing atrophic and fragmented muscle fibers are often found in deep fibromatoses (Fig. 2).

Immunophenotype

In both superficial and deep fibromatoses, vimentin and sometimes SMActin positive. Nuclear staining for B-catenin.



Fibromatosis, Cytological Findings, Fig. 2 Aggressive fibromatosis, desmoid: spindle cells with hyperchromatic nuclei which can be fusiform, round, or irregular and varying amount of poorly defined cytoplasm. MGG

Molecular Features

1. Superficial fibromatosis, gain of extra-chromosomes 7 or 8.
2. Deep fibromatosis, no consistent changes described.

Differential Diagnosis

Nodular fasciitis, fibroma of tendon sheath, low-grade fibrosarcoma.

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Fine Needle Aspiration Cytology

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Synonyms

Fine needle biopsy; Needle aspiration cytology; Thin needle aspiration biopsy

Definition

Fine needle aspiration cytology (FNAC) is a diagnostic technique based on analysis of the cells obtained by punction and aspiration with thin needle from tumors or lesions of various organs and parts of the body.

Principle

Cytologic evaluation of smears from aspirated cells.

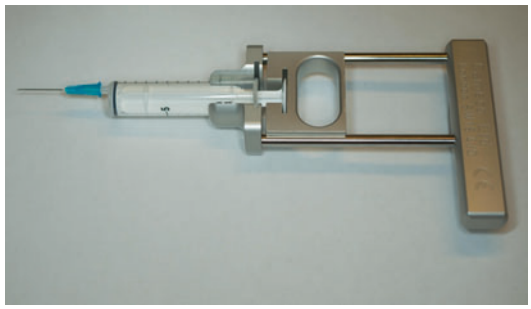
Methodology

Aspiration biopsy of palpable masses is performed with a thin needle (27 gauge/0.4 mm to 22 gauge/0.7 mm), fitted to a 10-ml syringe in a one hand grip syringe holder (Fig. 1). The target is usually fixed by pressing two fingers horizontally toward it which will immobilize the mass (Fig. 2). Large tumors are fixed between the thumb and the index finger. In most cases, one or two needle passes with several up and down strokes will provide enough cells for smears as well as cytospin preparations.

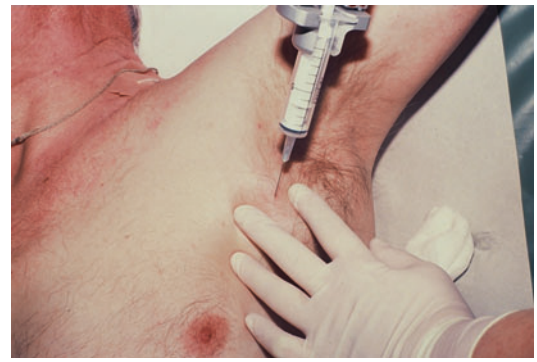
One part of the aspirated material is expressed onto a glass slide and smeared carefully with a spreader glass slide. The smears should be even and thin. Several matched smears can be produced from a small drop of aspirated cells using the "splitting technique." This is executed by touching the drop of aspirated material with the edge of a second glass slide several times. The small droplets are then transferred onto new slides and smeared. Using this procedure, it is possible to prepare several matched slides from only one aspirate (Fig. 3).

Quality Aspects

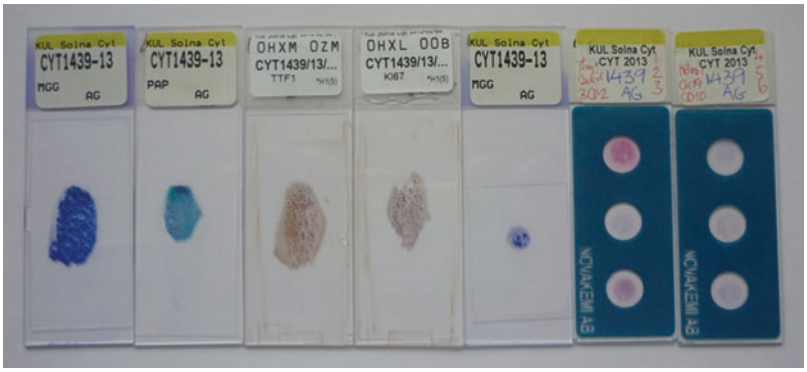
In experienced hands, FNAC has a sensitivity and specificity close to that of histopathology. It is of importance to point out that to achieve such a high diagnostic accuracy, all aspects of the procedure have to be performed with a maximum of skill. In the first place, the needling of the lesions has to be accurate to obtain representative cells. Secondly, the aspirated cells have to be processed to produce monolayer smears without crush artifacts. In some cases, a part of the aspirated cells should also be used for ancillary techniques such as immunocytochemistry, flow cytometry, bacteriology, and molecular biology (Fig. 3). Thirdly, the evaluation of the cytologic smears and immunologically stained material requires special training. All these steps will require supervised training since some aspects of the procedure will be difficult to acquire from textbooks.



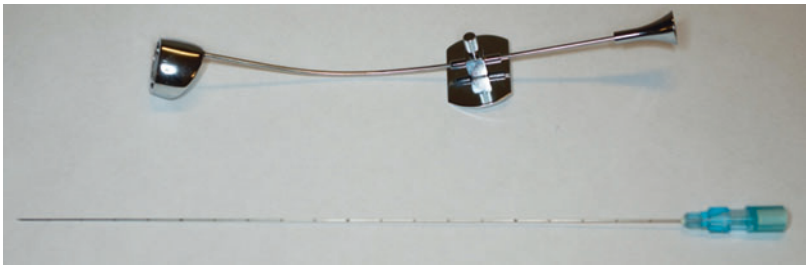
Fine Needle Aspiration Cytology, Fig. 1 One hand grip syringe holder (Franzén's pistol) with 10 ml syringe and a 23 gauge/0.6 mm needle



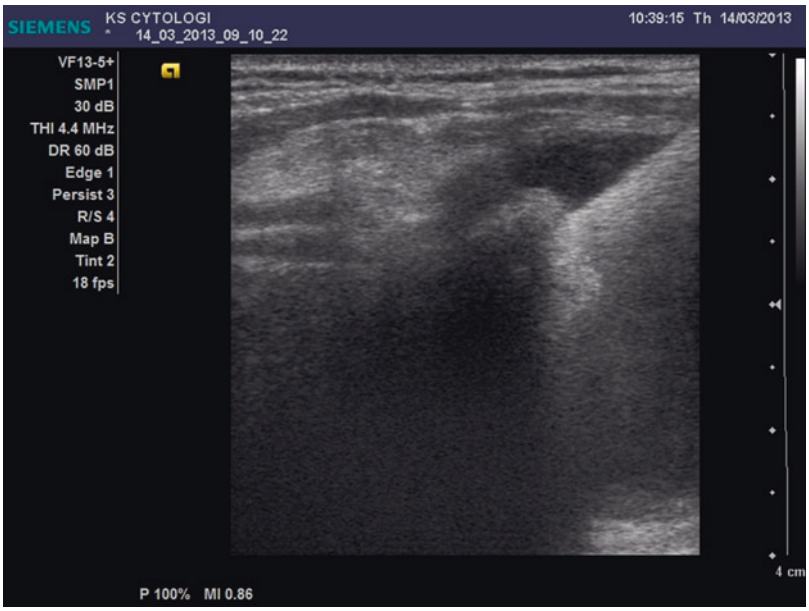
Fine Needle Aspiration Cytology, Fig. 2 FNA biopsy from a palpable axillary mass



Fine Needle Aspiration Cytology, Fig. 3 FNA smears stained with MGG, Papanicolaou, immunoperoxidase and cytospin preparations stained with MGG and alkaline phosphatase



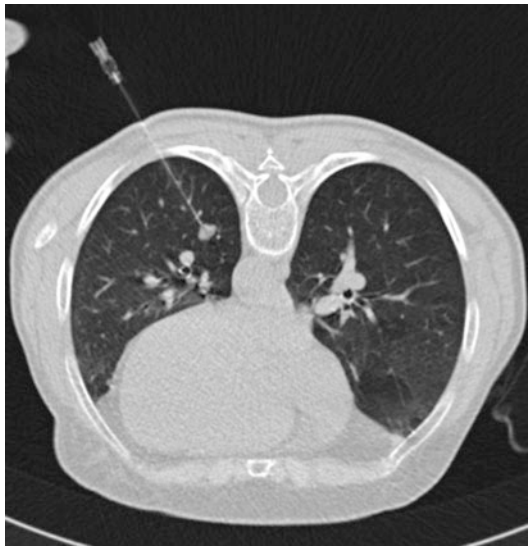
Fine Needle Aspiration Cytology, Fig. 4 Guide and needle (23 gauge) for biopsy of the prostate



Fine Needle Aspiration Cytology, Fig. 5 Ultrasound guided FNA biopsy of a lesion in the thyroid

Applications

The Franzen needle guide for prostate allows sampling from lesions that are palpable with the tip of the index finger through rectum (Fig. 4). Intrapelvic lesions can be also reached through rectum or transvaginal and samples taken with the Franzen guide with fine needle aspiration.



Fine Needle Aspiration Cytology, Fig. 6 CT guided FNAC of a small lung tumor

In the same way, para-pharyngeal masses, as well as adenoid or tong base lesions can be sampled.

Nonpalpable lesions can be identified and sampled with ultrasound and CT guidance (Figs. 5 and 6).

Lesions that are difficult to reach in the mediastinum, lung, retroperitoneum, pancreas, stomach, abdominal lymph nodes, and adrenal gland can be sampled by endoscopic ultrasound-guided fine needle aspiration.

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Flow Cytometry on FNA Material

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Synonyms

Pulse cytophotometry

Definition

Flow cytometry (FC) is a mean of measuring certain physical and chemical characteristics of cells or particles as they pass, as a fluid stream, through a laser light beam. The term "flow cytometry" derives from the measurement (meter) of single cells (cyto) as they flow through multiple detectors.

Principle

A flow cytometer is made up of three main systems: fluidics, optics, and electronics. The fluidics system transports particles in a stream through the laser beam for examination. The optic system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors. The electronic system converts the detected light signals into electronic signals that can be processed by the computer.

Methodology

A cell suspension is prepared and treated with a single or multicolored fluorochrome-tagged antibody with the aim of staining the cells which will pass in front of a laser beam. The laser beam hits the cell that absorbs the light and emits a fluorescence which will vary based on cell type and that can be measured. The electrical pulses that result from the emitted fluorescence are processed by a series of linear and log amplifiers. Logarithmic amplification is most often used to measure fluorescence in cells, expanding the scale for weak signals and compressing the scale for "strong" or specific fluorescence signals.

Data are then displayed in a computer in dot plots or histograms. Subpopulations can be studied gating regions of the histogram.

Quality Aspects

Flow cytometry can detect DNA content in human cancer and it can detect intracellular (various cytokines) or cell surface antigens (cluster of differentiation, CD markers). It can also evaluate the volume and morphological complexity of the cells, it can perform chromosome analysis, as well as evaluate protein expression and localization. Comparing with other methods that can also be applied to cytology, with similar purposes, such as immunocytochemistry, FC permits the correct evaluation of clonal lymphoid populations, even when scarcely represented, or even when blended in a polyclonal population; it permits the evaluation of multiple antibodies coexpression in a single cell. FC analysis is not subjective and results can be measured, and consequently it is not dependent on interobserver/intraobserver variability. Its main drawback is the blind method for which the morphologic detail of what is being measured or characterized cannot be appreciated. In lymphoma diagnosis, FC should never be evaluated alone, and it should always be a complementary tool in a multidisciplinary approach together with cytomorphology and clinical data.

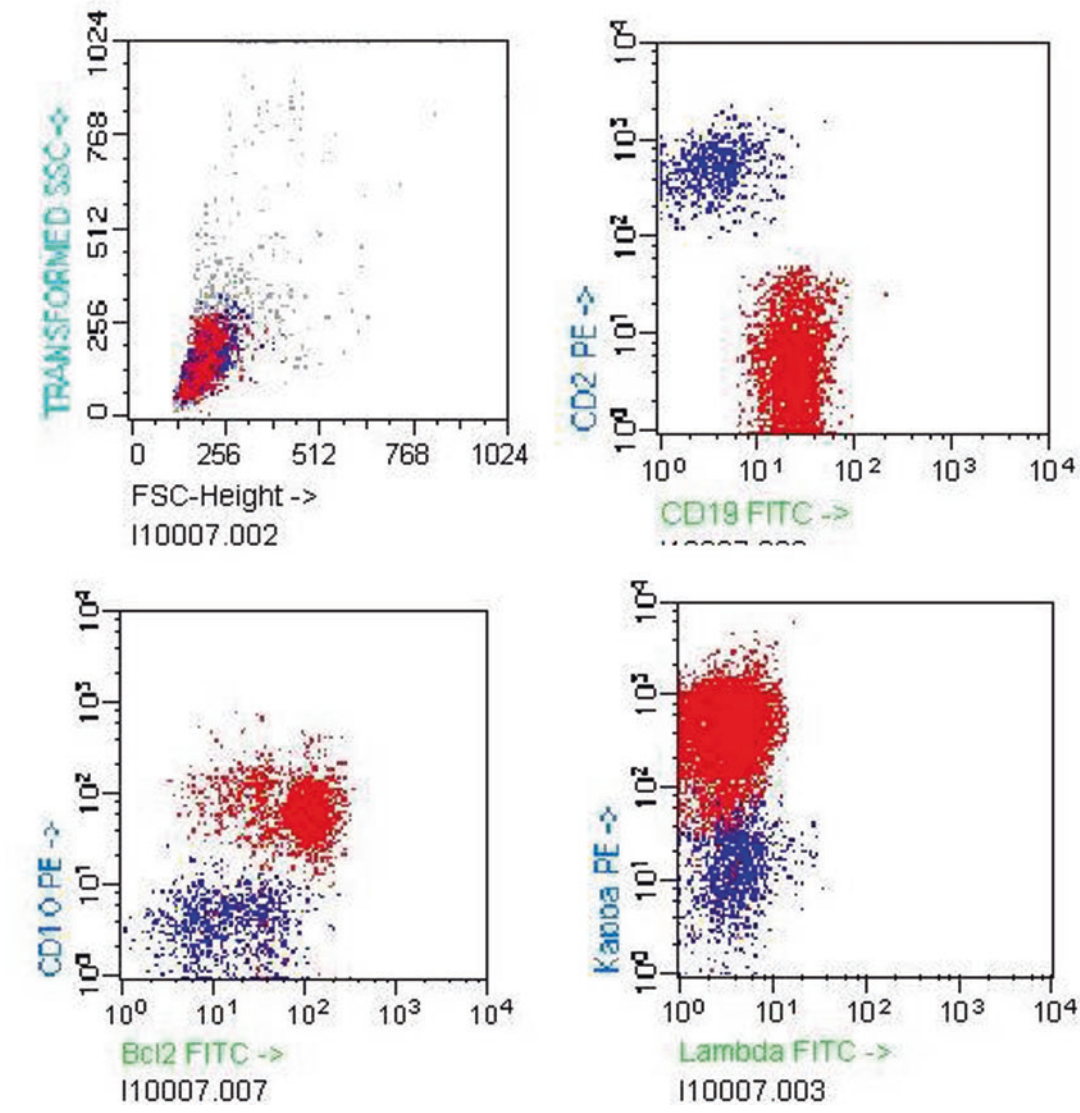
Applications

Application is wide covering molecular biology, pathology, immunology, plant biology, and marine biology. When applied to medicine, it can be used for immunophenotyping leukemia and lymphomas, detecting minimal residual disease, evaluating RNA content in reticulocytes, or diagnosing immune deficiencies. It is also used to evaluate platelet count and to measure cell surface proteins. Apoptosis can also be evaluated through FC.

It can also be helpful in evaluating the efficacy of cancer chemotherapy. In microbiologic studies, its application to bacteria, fungi, parasites, and viruses detection has been referred.

FC immunophenotyping is now an established and indispensable laboratory tool for the diagnosis, classification, staging, and monitoring of hematologic neoplasms. The evaluation of cytomorphology and immunophenotype represent the major criteria that are needed to diagnose and subclassify lymphomas nowadays (Dey 2006; Barroca et al. 2008).

When applied in lymphoma diagnosis, it helps in distinguishing neoplastic cells from benign/reactive lymphoid populations. It permits the identification of minor neoplastic populations based on phenotyping and characterizing them. The main advantages of applying this technique in the cytological diagnosis of lymphomas are due to the fact that it provides a rapid sensitive and quantitative measurement of monoclonal antibodies raised against cytoplasm or nuclear lymphocytic antigens even when a minor quantity of cellular material is available (Fig. 1). FC can also detect other neoplasia using markers like CD56 (e.g., rhabdomyosarcoma, ▶ neuroblastoma, or neuroendocrine tumors; pheochromocytomas; pancreatic solid pseudopapillary tumor; synovial sarcoma; and nephroblastoma), CD99 in the Ewing family tumors (EFT) and in some rhabdomyosarcomas, and CD117 in germinal tumors (seminomas/dysgerminomas), EFT, gastrointestinal stromal tumors, and adenoid cystic carcinoma of salivary gland (Table 1).



Flow Cytometry on FNA Material, Fig. 1 FC obtained from a fine-needle aspiration cytology of a lymph node with follicular lymphoma. (*Blue* T lymphocytes, *Red* B lymphocytes). All B cells are CD10+ and kappa light chain positive. There is also an overexpression of bcl2

Flow Cytometry on FNA Material, Table 1 Flow cytometry diagnostic parameters in non-hematologic neoplasms

	CD45	CD56	CD99	CD90	EpCam(epithelial glycoprotein 2)
Neuroblastoma	–	++++	–	++	–
EFT	–	++	++++	++++	–
Rhabdomyosarcoma	–	++	–	+/–	–
Small cell carcinoma	–	++	–	–	++

DNA analysis is the second most important application of flow cytometry. The measurement of DNA content (ploidy) serves to calculate the percentage of a cell population in each phase of the classic cell cycle. The percentage of cells in the S-phase gives an indication of the proliferative activity of that cell population.

This application has been carried out coupled with cytology as such as in detecting cancer cells in urine specimens (Bakhos et al. 2000). Ploidy study is also very important in the prognosis evaluation of neuroblastomas. Neuroblastomas hyperdiploid profile is associated with earlier stages of disease, better response to chemotherapy, and a more favorable prognosis than a diploid profile.

Detection of minimal residual disease is another major application of FC. Patients with acute leukemia are considered to be in remission when bone marrow samples contain less than 5% of neoplastic cells. Cytomorphologic examination of bone marrow (BM) aspirates is not sensitive enough to detect residual leukemic and neuroblastoma cells. Flow cytometric methods can detect far lower levels of disease, which can be important in the clinical management of leukemia and neuroblastoma.

Diseases resulting from primary immunodeficiencies, usually found in infants and young children, result from defects in T cells, B cells, granulocytes, or monocytes. Flow cytometry can also be used to detect functional abnormalities in leucocytes, evaluating the presence of missed or impaired surface or cytoplasmic proteins.

Examples

See Table 1

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Follicular Tumor, Cytological Findings

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Synonyms

Follicular neoplasm

Definition

Follicular tumors concern proliferation of well-differentiated follicular cells and include follicular adenoma as well as follicular carcinoma. This category should not include the follicular variant of papillary carcinoma (FVPC), but due to the frequent lack of atypia suggestive for papillary carcinoma in this variant, this may happen. Hürthle cell tumors are excluded from this group (see ▶ Hürthle Cell Tumors, Cytological Findings) as well as the hyperplastic (adenomatoid) nodules.

Clinical Features

- **Incidence**
  The estimated incidences for follicular tumors are 2% for women and less than 1% for men.
- **Age**
  The prevalence of the follicular tumors increases with age, reaching to about 50% after 60. The risk of cancer is higher for people less than 30 and over 60.

- **Sex**
  The sex ratio is F: M: 2–4:1. The risk of cancer is twice as higher for men than for women.
- **Site**
  The tumors may be found anywhere in the thyroid gland.
- **Treatment**
  The treatment is extremely variable. Follicular adenomas may be removed or not, depending on their size at the time of diagnosis as well as during the follow-up, depending on if they remain stable or increase in size, their ultrasonographic characteristics, the fine needle aspiration (FNA) results, and the patient's wishes. Follicular carcinomas have to be operated, usually by total thyroidectomy, followed or not by radioiodine therapy.
- **Outcome**
  The prognosis is excellent for follicular adenoma, since it is a benign tumor. It is extremely variable for follicular carcinoma, depending on the patient's age, the size and the histological stage of the tumor as well as the existence or not of lymph nodes metastases; age higher than 45 years, size higher than 4 cm; and pT3 and metastases represent adverse factors. Ten-year survivals for low-, intermediate-, and high-risk groups are 98%, 88%, and 56%, respectively.

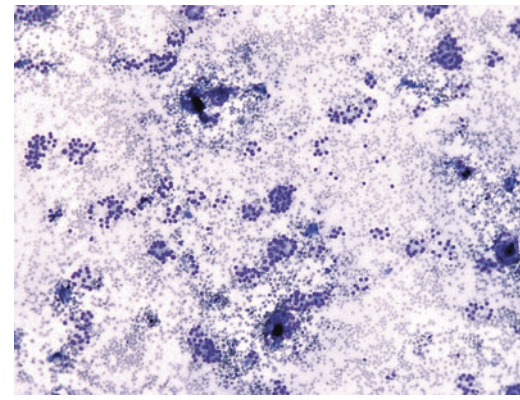
Macroscopy

Follicular adenoma is usually a round or oval tumor, well-delimited by a very thin and quite unapparent capsule. Its color is variable, depending on the quantity of colloid, gray-white or brown or tan. Some hemorrhagic foci and cysts may be observed. Follicular carcinomas are also round or oval tumor, often gray-brown or brown. Invading tumors present irregular borders and invasion of the adjacent thyroid gland; the minimally invasive follicular carcinomas are difficult to distinguish from follicular adenoma; in such cases, a thick capsule may be helpful to suspect a malignant nodule.

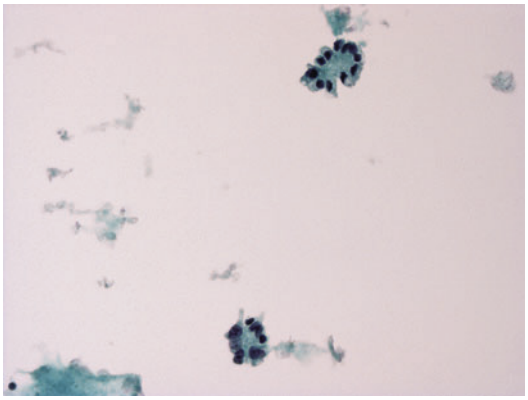
Cytological Findings

Follicular tumors or neoplasms share some similar cytological features which explain why it is cytologically so difficult to distinguish one entity from the other. Cellularity, microfollicles, nuclear size, and colloid represent the four criteria on which the diagnosis is based (Fig. 1). A normal cellularity, microfollicles with normal nuclei, and colloid drops are more in favor for a benign tumor; inversely, a high cellularity, numerous microfollicles with enlarged or atypical nuclei, and lack of colloid are really suspicious for malignancy. Nevertheless, all these criteria are not sufficient to assert the diagnostic of benign or malignant follicular tumors. For these reasons, all these cases are classified as "Follicular neoplasm" in the Bethesda terminology 2009 (BSRTC) and a lobectomy is required for single nodules. In some cases, a diagnosis of follicular adenoma may be cytologically done: the association of a normal cellularity with very few microfollicles, normal round nuclei in the follicular cells, and colloid are usually signs of a macrofollicular benign adenoma.

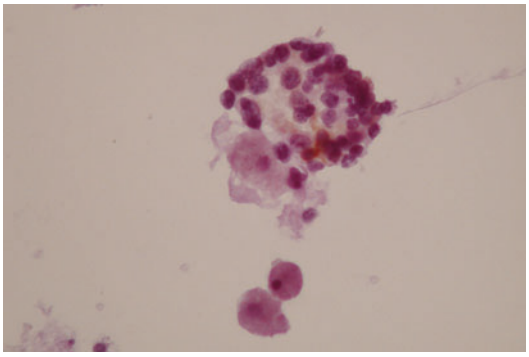
On liquid-based cytology, the criteria differ slightly: Cellularity is no more a significant diagnostic criteria; colloid may be lacking in true benign tumor; microfollicular arrangement and



Follicular Tumor, Cytological Findings, Fig. 1 High cellularity, numerous microfollicles, and lack of colloid (conventional slide; MGG x25)



Follicular Tumor, Cytological Findings, Fig. 2 Microfollicles but nuclear atypia suggestive for papillary carcinoma. Features of a papillary carcinoma – follicular variant (LBC; Hologic®; Papanicolaou staining ×40)

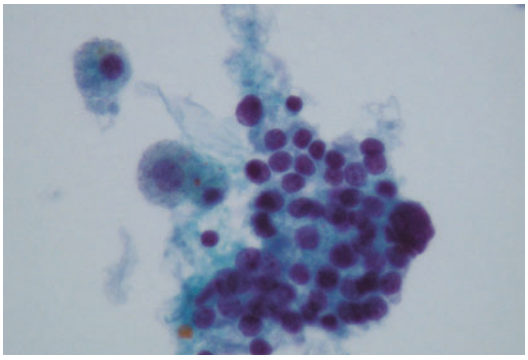


Follicular Tumor, Cytological Findings, Fig. 3 HBME1 essentially negative (LBC; Hologic®; ×40) favor benign

nuclear size remain the "key diagnostic criteria" (Fig. 2).

Immunophenotype

The neoplastic cells of the benign and malignant follicular tumors are both positive for many antibodies such as HBME1 (Fig. 3), CK19, Galectine 3, CD44v6, cycline D1, etc., but with a percentage of positive cells usually lower in benign tumors and higher in malignant ones.



Follicular Tumor, Cytological Findings, Fig. 4 Microfollicles included in the sheets of follicular cells and lack of colloid (LBC; Hologic®; Papanicolaou staining ×40)

Therefore, some cytopathologists have tried to apply immunocytochemistry to follicular neoplasms. Even if the immunocytochemical profile is not clear-cut in all cases, it may be helpful in about 50% of these tumors.

Molecular Features

PAX8-PPARγ fusion is found in 25–50% of follicular carcinoma and rarely in follicular adenoma; K-RAS mutation has been described in about 50% of follicular carcinoma and only seldom in adenoma.

Differential Diagnosis

Follicular tumors are relatively easy to recognize cytologically – essentially due to their microfollicular arrangement. The main differential diagnosis concerns the follicular variant of papillary carcinoma (FVPC); nuclear atypias such as nuclear grooves, elongated nuclei, and/or pseudoinclusions should move these cases into the BSRTC category "suspicious for malignancy: suspicious for papillary carcinoma-variant follicular" (Fig. 4). Nevertheless, these nuclear atypias are sometimes very discrete and may not be present.

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Fungi Infections, Cytological Findings

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Synonyms

Granulomatous lymphadenitis of fungus etiology

Definition

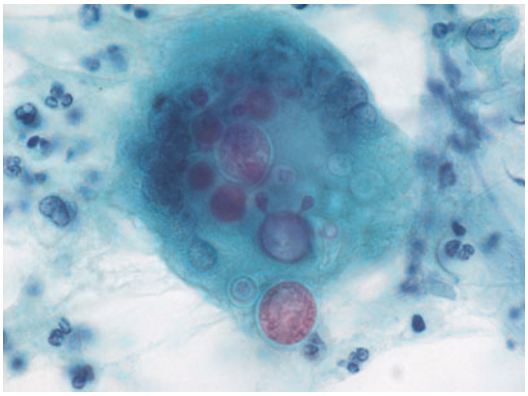
Fungal infections have significant manifestations in the lymph nodes as a disseminated infection or primary infections spread to local or regional nodes. Most commonly seen in immunosuppressed patients, especially those with HIV/AIDS or post transplantation.

Clinical Features

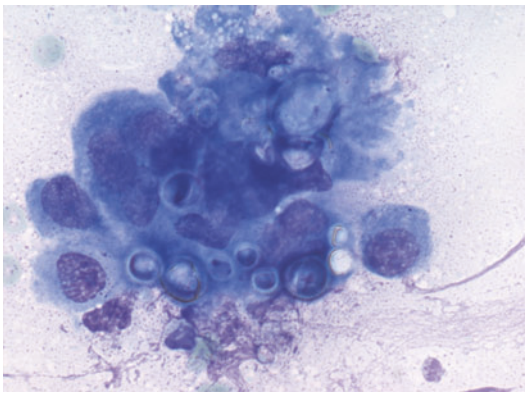
- **Incidence**
  Varies between continent, countries, and regions. *Cryptococcus neoformans* has a worldwide distribution and is endemic in soil. *Blastomyces dermatitidis*, *Histoplasmosis*, and *Coccidioides immitis* are most often seen in the United States, but cases are also seen in Central and South America. *Paracoccidioides brasiliensis* is endemic in South and Central America.
- **Age**
  All ages can be affected.
- **Sex**
  No sex predilection.
- **Site**
  Lymph nodes are the most common site but spleen and various parenchymatous organs may also be affected.
- **Treatment**
  Fluconazole, itraconazole, ketoconazole, anphotericin B, trimethoprim-sulfamethoxazole, and pentamidine are the most common agents used.
- **Outcome**
  Many patients are cured, but later relapses occur especially in HIV/AIDS patients.

Microscopy

Acute inflammatory reaction with the presence of neutrophils, cell debris, and necrotic material. Epithelioid histiocytes forming granulomas and multinucleated giant cells are often seen. The causing organisms can often be demonstrated by special staining such as PAS, mucicarmin, and Gomori methenamine silver but specific identification may require culture (Figs. 1–3).



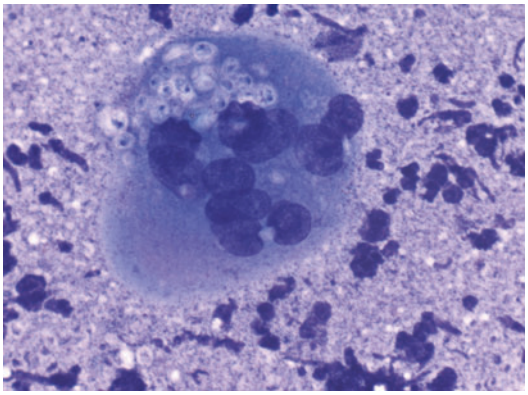
Fungi Infections, Cytological Findings, Fig. 1 Paracoccidioidomycosis. Smear from lymph node aspirate shows a multinucleated giant cell with yeast forms with thick wall and several budding daughter yeasts. Papanicolaou



Fungi Infections, Cytological Findings, Fig. 2 Paracoccidioidomycosis. Same case as Fig. 1 stained with MGG shows histiocytes, giant cell, and yeast forms. MGG

Molecular Features

PCR with specific primers is used to conclusively identify some of the fungi.



Fungi Infections, Cytological Findings, Fig. 3 Histoplasmosis. Smear from aspirate shows necrotic background with cell debris and a multinucleated giant cell with small yeast organisms. MGG

Differential Diagnosis

Granulomatous lymphadenitis of other etiology and necrotic lymphadenopathy

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G

Germ Cell Neoplasias, Cytological Findings

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Synonyms

Choriocarcinoma (chorionepithelioma); Embryonal carcinoma (undifferentiated malignant teratoma); Seminoma-testis/digerminoma-ovaries and polyembrioma; Teratoma; Yolk sac tumor (endodermal sinus tumor)

Definition

Germ cell tumors originate from germ cells and can arise whether in ovaries, in the testicle, or in other locations. Human germ cells during embryo development migrate along to the genital ridges to become incorporated in future gonads. The failure of this migration leads to the persistence of ectopic lost germ cells that can give origin to extragonadal germ cell tumors. Extragonadal tumors are rarer than gonadal tumors, unless for female sacrococcygeal teratomas in newborns.

Germ cell tumors are a heterogeneous group of neoplasms that include seminomatous/germinomatous and non-seminomatous/germinomatous tumors. This last group includes teratoma, yolk sac tumor, embryonal carcinoma, and choriocarcinoma. In a considerable number of cases, these different subtypes coexist, and these tumors are then called as mixed germ cell tumors.

Clinical Features

- **Incidence**
  Incidence varies according to age and to the tumor location of the affected patient.
- **Age**
  The age of presentation differs according to gender and location. The incidence of germ cell tumors varies according to age. Teratomas are the most common tumor in newborns, while malignant germ cell tumors are scarce in this age, affecting mainly postpuberty children. In neonates, infants, and in children younger than 4 years, the majority of germ cell tumors are sacrococcygeal teratomas, and most of them are mature dermoid cysts. Malignant and immature teratomas occur later in life around 15–30 years old. Yolk sac tumors appear most frequently around the age of 3; embryonal carcinomas and choriocarcinomas occur from adolescence to adult age and can appear in patients aged 30–40 years (Choi et al. 2004; Babu et al. 1996; Kumar et al. 1991).

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4

Dysgerminoma and seminoma occur in adults around 40–50 years old.

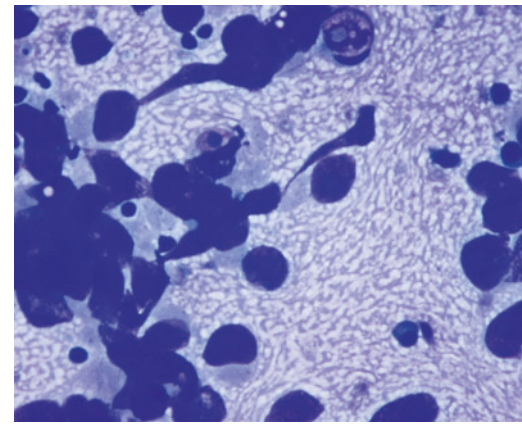
- **Sex and Site**
  Despite their name germ cell tumors can occur both within and outside the gonads (testicle 12% and ovary 25%). The occurence of these tumors in the ovary and in the testis is much more frequent, but they can appear intracranial in 5% of the cases, affecting the pineal gland, in a suprasellar location; inside the mouth, in the neck, and mediastinum in 18% of the cases; in the sacral region in 40% of the cases; and in the pelvis.
  In the United States sacrococcygeal teratoma occurs in 1 in 30,000–70,000 live births. In males they can be bilateral in 1–2% of the cases, and when this happens they are usually seminoma. In undescended testes, bilateralism is higher and arrives up to 15% of the cases.
- **Treatment**
  The first step to start treating a germinative tumor is to separate benign germinal tumors (mature teratomas) from malignant germinal tumors, seminomatous and non- seminomatous germinal tumors (NSGT). Tumor treatment is also determined by the tumor staging, age, and location. Benign germ cell tumors are treated with surgery.
  In malignant germ cell tumors and in low-stage disease, surgery is indicated. Chemotherapy is used as primary therapy in advanced disease (stage IIC and III) or as adjuvant therapy in low-stage disease.
- **Outcome**
  Germ cell tumors behave differently according to gender, age, and location.
  In the ovary, teratomas represent more than 95% of all germ cell tumors. These tumors are frequently benign regardless of age.
  Testicular teratomas arising in the prepubertal age are most often benign, but in postpubertal testis these tumors should be considered malignant even if they have a bland-looking histology.
  Behavior is also intimately connected with histology. Yolk sac tumors, embryonal carcinoma, and germinomatous tumors are considered malignant. Teratoma is considered in high frequency of the cases as a benign tumor, although prognosis depends on the presence or absence of immature or malignant elements that may be present in its midst.
- **Macroscopy**
  See histology template.

Microscopy

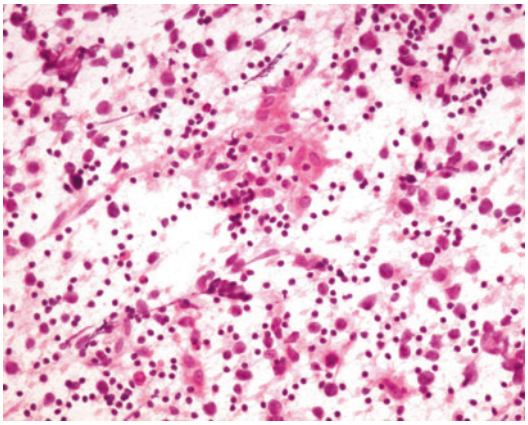
Despite the common cell origin, germ cell tumors have varied histologic and cytologic patterns and are described separately.

Seminomas/Germinomas

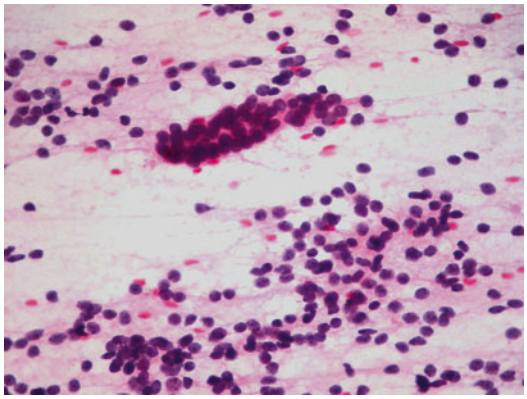
Cytologic smears of these tumors generally bear a high to moderate cellularity. The most common presentation is that of a heterogeneous dispersed cell population, composed of small lymphocytes (nontumoral), histiocytic epithelioid cells (nontumoral), and malignant large single cells that lay on a hemorrhagic and tigroid background (Fig. 1). Neoplastic cells are characterized by large monotonous nuclei with scant to moderate cytoplasm. The nucleus is centrally placed and has a coarsely clumped chromatin, with a single prominent eosinophilic nucleolus (Orell et al. 1999; Gupta et al. 2008). Cytoplasm is fragile and when preserved it is finely vacuolated due to



Germ Cell Neoplasias, Cytological Findings, Fig. 1 Seminoma – remark the presence of a tigroid background, with lymphocytes and single dispersed and large neoplastic cells (Giemsa 400x)



Germ Cell Neoplasias, Cytological Findings, Fig. 2 Seminoma – in a background with small dispersed lymphocytes, one can see a small epithelioid granuloma and large characteristic neoplastic cells with prominent nucleoli (H&E 100x)



Germ Cell Neoplasias, Cytological Findings, Fig. 3 Yolk sac tumor – a mucopolysaccharide-rich fluid is noticed as a thin eosinophilic layer in hematoxylin/eosin in the background. Neoplastic cells lay in tridimensional clusters. Nuclei are large and cytoplasm is abundant and vacuolated with even signet-ring-like appearance (H&E 200x)

the richness in glycogen. Leakage of glycogen through this fragile cytoplasm confers these smears a characteristic and recognizable tigroid background in Giemsa stains. Lymphocytes and sometimes polymorphs are present and represent a response of the host. In some cases small epithelioid granulomas and multinucleated giant cells are also present (Fig. 2). Mitoses as well as necrotic foci are common.

Yolk Sac Tumor/Endodermal Sinus Tumor

Characteristically fine needle aspiration material of a yolk sac tumor bears a mucoid viscous translucent fluid material, rich in mucopolysaccharides. This translucid film is perceptible as a thin eosinophilic layer in hematoxylin/eosin stained smears, as a metachromatic layer in Giemsa and as an homogenous green layer in Papanicolaou stained smears. Smears have large to moderate cellularity, and cells lay single or in aggregates, sometimes crossed by capillaries and basement membrane-like material (proteoglycan, laminin, and collagen type IV – *Reichert's membrane)*. Some authors also refer the presence of papillary groups and Schiller-Duval bodies. The cells are polygonal and have an epithelial appearance containing large obvious malignant nuclei with coarse chromatin and evident nucleolus (Fig. 3) (Orell et al. 1999; Gupta et al. 2008). Cytoplasm can be scant or else, abundant and vacuolated with even signet-ring-like appearance and containing mucoid material. In some cases eosinophilic hyaline globules are seen in intra- or extracellular location. These globules are PAS, alpha-fetoprotein, and alpha-1-antitrypsin positive. In the smear background, necrotic material can be evident even though less evident than in embryonal carcinoma.

Embryonal Carcinoma

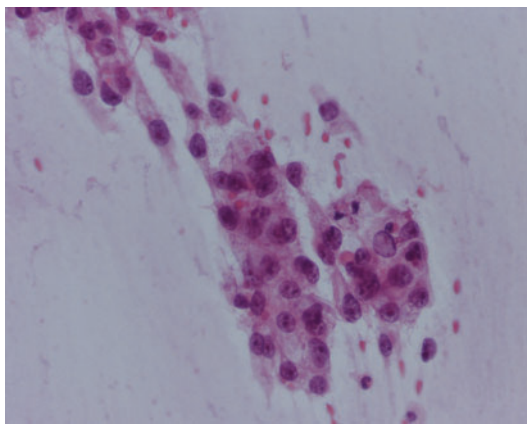
These neoplasms are composed of large polygonal cells and abundant necrotic material, simulating a high-grade adenocarcinoma. However, there is no mucous production by neoplastic cells. The cells dispose themselves in glandular structures, papillae, or solid aggregates (Gupta et al. 2008). The cells of embryonal carcinoma are large with atypical huge nuclei and prominent eosinophilic nucleoli. Anisonucleosis can be marked with the presence of bizarre forms (Orell et al. 1999; Gupta et al. 2008). The nucleus, generally unique and centrally placed, has an irregular membrane and one or more prominent nucleoli. The cytoplasm is scant and a high N/C relation is patent. Mitoses and apoptotic cells are numerous.

Choriocarcinoma

Cytologic pattern of choriocarcinoma is that of a high pleomorphic smear with extensive hemorrhage and necrosis. Aspiration of pure cases is rare, and the author's personal experience is quite limited. Cytological findings in case of choriocarcinoma in different sites such as testis, mediastinum, and breast are described. Mononucleated and polynucleated and pleomorphic cells are present. Most mononucleated cells occur single or in cohesive groups and have high N/C ratio with solitary dark nuclei and prominent nucleoli (Orell et al. 1999; Naniwadekar et al. 2009). Cytoplasm is vacuolated with indistinct borders.

Teratoma

Most teratomas are ovarian cystic benign teratomas. These mature teratomas are composed of mature elements, derived from ectoderm, endoderm, and mesoderm (Fig. 4). Mature teratomas are either cystic containing hair and sebaceous material or hard lesions containing calcifications or bone fragments. This makes aspiration difficult and poorly cellular. Sometimes cysts are aspirated being most of the time noninformative due to hypocellularity and poor epithelial representation. Willingly, when suspicion of a teratomatous lesion is raised, the patient should be sedated, as you will sometimes need to perform multiple passes till you have enough material that will permit you a confident diagnosis. This attitude is particularly relevant in children. If the sample is to be representative, in these lesions generally various types of mature tissues, (pavimentous and glandular epithelia, cylindrical cells, sometimes with a brush border, goblet cells, mesenchymal stroma, cartilage, adipose cells, skeletal and smooth muscle, bone, and glial tissue, among others are observed. Occasionally a component of uncommitted small round cell is appreciated being most of the time representative of neuroblastic tissue or even blastema. This event should call your attention for the possibility of an immature teratoma. However, the subclassification of the teratomas should be done only with the final examination of the entire specimen. Mature or immature teratoma classification has revealed useless once they share the same genetics and biologic potential independent of age. However, the recognition of yolk sac-like tumor or embryonal carcinoma or other malignant somatic cellular representation should be referred as it deeply affects outcome and treatment. Testicular teratomas in postpubertal patient should also be considered as malignant apart of its cytologic pattern, as they metastasize in 29% of the cases.



Germ Cell Neoplasias, Cytological Findings, Fig. 4 Teratoma – remark the presence of an epithelial mature cellular group and an immature component composed of single dispersed neuroblastic cells (H&E 100x)

Immunophenotype

See Table 1: Immunophenotype of germ cell tumors

Molecular Features

Testicular germ cell tumors can be considered into five molecular groups:

Type I – Infantile teratomas are diploid lesions with no i (12p). Pure yolk sac tumors of infancy are aneuploid and also lack i (12p).

Type II – Seminoma and non-seminoma of young postpubertal adults; these tumors have i (12p) and frequent loss of chromosome 13 or gains in chromosome 21.

Type III – Spermatocytic seminomas of elderly; these tumors have overrepresentation of chromosome 9.

Germ Cell Neoplasias, Cytological Findings, Table 1 EMA, epithelial membrane antigen; Anti-Pan cytokeratin (AE1/AE3); *CEA* carcinoembryonic antigen, *CD* cluster differentiation, *PLAP* placental alkaline phosphatase, *HCG* human chorionic gonadotropin, *OCT* optimal cutting temperature

	tEMA	AE1AE3	CEA	CD30	CD117	PLAP	Alpha-fetoprotein	Beta HCG	OCT 3/4	CD143
Seminoma/ germinoma	–	+/–		–/+ **In atypical types**	+	+	+/–	In syncytiotrophoblastic cells	+	+
Yolk sac tumor	+	+	+	–	–	+	++ **(sometimes focal)**			
Embryonal carcinoma	–	+	–	++	–	++	+			

Type IV – Dermoid cysts have a diploid genotype.

Type V – Gestational trophoblastic tumors are diploid.

Differential Diagnosis

Differential diagnosis depends on the clinical setting and depends on the type of the tumor in question. Extragonadal locations like mediastinum, retroperitoneum, sacrococcygeal, and supraclavicular can be the primary site or the first clinical presentation of metastatic disease.

Differential diagnosis of seminoma and germinoma should include embryonal carcinoma, yolk sac tumor, adenocarcinoma, ▶ melanoma, thymic carcinoma (in mediastinal lesions), Hodgkin disease, T-cell-rich B-cell lymphoma, and large cell lymphomas. Embryonal carcinoma, yolk sac tumor, and adenocarcinoma have all together very cohesive patterns. Cells dispose in glandular and papillary aggregates, at least focally. Other details like mucoid background, hyaline globules, metachromatic basement membrane, and lack of pleomorphism help in the diagnosis of yolk sac tumor. Melanoma shares with seminoma the single cell arrangement. Nuclear pleomorphism is more evident in melanoma. Binucleated cells with opposed nuclei ("divorced cells") are a hallmark. Lymphocytes, small granulomas, and tigroid background are not present in melanoma. The presence of melanin pigment in the tumor cells drops the diagnosis of seminoma or germinoma. In mediastinum tumors, thymic carcinoma raises some diagnostic disturbance, especially lymphoepithelioma like carcinoma. Once again the presence of tigroid background and granulomas point out to seminoma. In Hodgkin disease and non-Hodgkin lymphomas, differential diagnosis with seminoma should be placed. Ocassionally in lymphomas the presence of a dual population of malignant lymphocytes and host-reactive lymphocyte population coexists, and cytological aspects overlap those of seminoma and germinomas. This is particularly evidentt in Hodgkin disease, and T-cell-rich B-cell lymphoma. In seminoma, the presence of a tigroid background and absence of lymphoglandular bodies favor the diagnosis of seminoma. Like in seminomas small granulomas can also be present in Hodgkin or non-Hodgkin lymphomas. Large B-cell lymphoma, immunoblastic type, has also a dispersed cell pattern as well as many nuclei similarities to seminoma, but does not present a tigroid background. Immunostains are generally discriminative.

Embryonal carcinoma simulates a poorly differentiated carcinoma. This is mainly pertinent in extragonadal locations, as in cervical or supraclavicular location.

The main problem posed in cytological smears of teratomas is the misunderstanding of local normal/reactive contaminant structures like bone, cartilage, adipose tissue, or epithelial components. Cytologists should perform themselves the biopsy or have total confidence on the person who performs it, so that they can assure the representativeness of the sample.

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Giant Cell Tumors of the Bone, Cytological Findings

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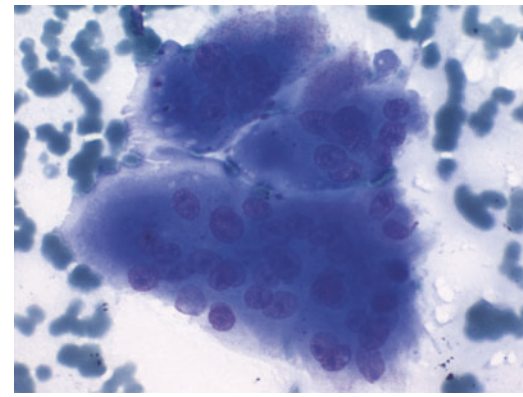
Synonyms

Osteoclastoma

Definition

Giant cell tumor (GCT) is a locally aggressive benign neoplasm composed of monomorphic mononuclear cells interspersed with uniformly distributed large, osteoclast-like giant cells (Fig. 1).



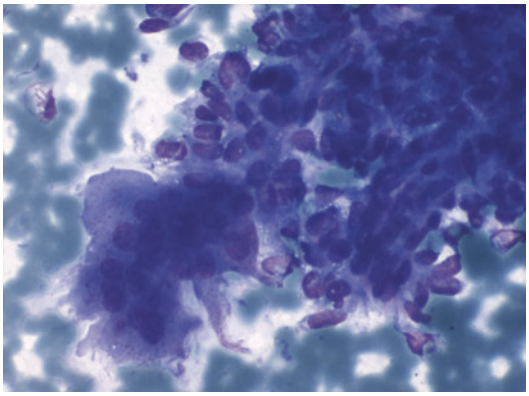
Giant Cell Tumors of the Bone, Cytological Findings, Fig. 1 Giant cell tumor of the bone: multinucleated giant cells with numerous monomorphic nuclei with prominent nucleoli. MGG

Clinical Features

- **Incidence**
  4–5% of all primary bone tumors.
- **Age**
  20–45 year of age.
- **Sex**
  Slight female predominance.
- **Site**
  GCT typically affects the epiphyseal region of long bones: distal femur, proximal tibia, distal radius, and proximal humerus. Pelvis, sacrum, and tubular bones of the hands and feet can be involved.
- **Treatment**
  Curettage, supplemented with bone grafting, cementation, cryotherapy, or instillation of phenol. Block excision for lesions in small bones.
- **Outcome**
  Local recurrence occurs in approximately 25% of patients usually within 2 years. Pulmonary metastases occur in 2% of patients 3–4 years after primary diagnosis (Fig. 2).

Microscopy

GCTs are composed of a dual population of monomorphic mononucleated spindle cell often



Giant Cell Tumors of the Bone, Cytological Findings, Fig. 2 Giant cell tumor of the bone: a multinucleated giant cell attached to a tissue fragment of monomorphic mononuclear tumor cells. MGG

densely packed in tissue fragments in clusters and giant multinucleated cells with sharply demarcated cytoplasm.

Immunophenotype

Tumor cells are positive for vimentin and CD68.

Molecular Features

GCT of the bone is characterized by the presence of a phenomenon defined as "telomeric associations" with telomere length reduction. The most common telomeres affected include 19q, 11p, 13p, 14p, 15p, 20q, 18p, and 21p.

Differential Diagnosis

Osteoblastoma, chondroblastoma, giant cell-rich osteosarcoma, solid aneurismal bone cyst, brown tumor of hyperparathyroidism, and giant cell reparative granuloma.

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Granular Cell Tumor, Cytological Findings

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Synonyms

Abrikossoff's tumor; Granular cell myoblastoma; Granular cell nerve sheath tumor; Granular cell schwannoma

Definition

Granular cell tumors are rare, usually benign slow-growing soft tissue tumors derived from Schwann cell.

Clinical Features

- **Incidence**
  Relatively rare.
- **Age**
  Usually middle aged but can occur in all age groups.

- **Sex**
  More common in females.
- **Site**
  All parts of the body most often in the head and neck.
- **Treatment**
  Surgery.
- **Outcome**
  Most cases are benign and do not recur. Malignant variant exist.

Microscopy

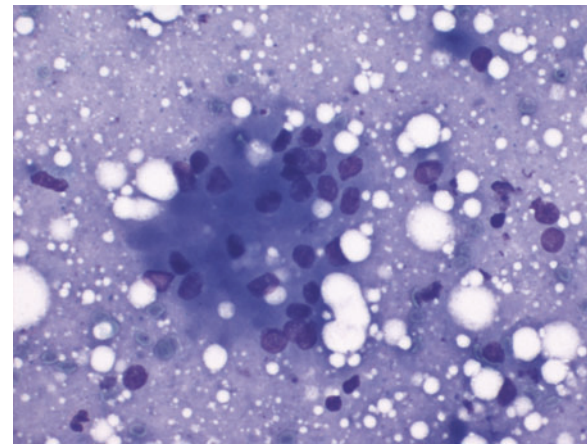
The cells have round or oval monomorphic nuclei, inconspicuous nucleoli, and abundant, often ill-defined granular cytoplasm. Clusters of varying sizes can be seen but dispersed cells and naked nuclei are common (Figs. 1, 2).

Immunophenotype

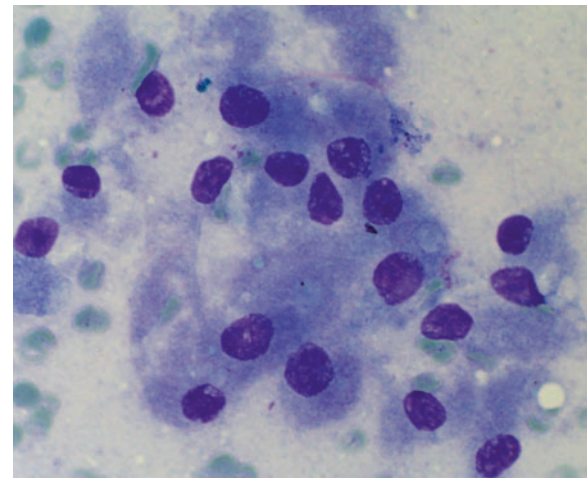
Vimentin, CD68, and S100 positive.

Molecular Features

No specific karyotype characteristics have been described.



Granular Cell Tumor, Cytological Findings, Fig. 1 Granular cell tumor: loose cluster of tumor cells with blue indistinct cytoplasm and round nuclei. The background shows cytoplasmic debris and few naked round nuclei. MGG



Granular Cell Tumor, Cytological Findings, Fig. 2 Granular cell tumor: cells with round or oval nuclei and a rich poorly delineated grey-blue finely granulated cytoplasm. High magnification MGG

Differential Diagnosis

Rhabdomyoma and hibernoma.

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Granulomatous Lymphadenitis, Cytological Findings

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Definition

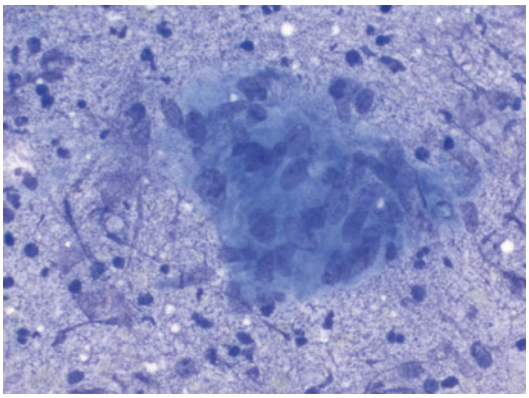
A specialized immune reaction in which macrophages of epithelioid type, various inflammatory cells, and multinucleated giant cells participate. Inciting agents are certain infectious organisms such as *Mycobacterium tuberculosis* or *avensis*, fungi, viruses. Additional causes are systemic diseases, such as sarcoidosis and rheumatoid arthritis, and foreign bodies.

Clinical Features

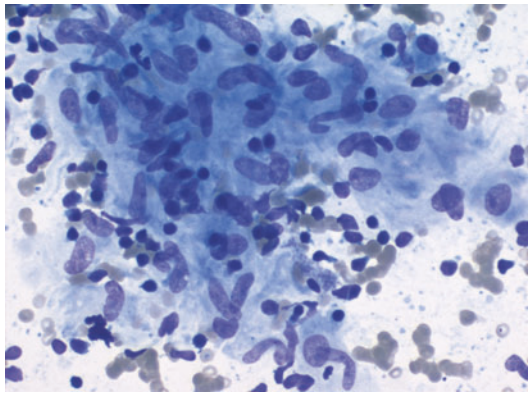
- **Incidence**
  No exact figures exist, but in Western countries, it is relatively rare.
- **Age**
  All ages are affected.
- **Sex**
  There is no sex predilection.
- **Site**
  Lymph nodes are the most common sites, but spleen and various parenchymatous organs can also be affected.
- **Treatment**
  Varies with the underlying cause.
- **Outcome**
  Depends on the underlying cause.

Microscopy

FNA smears show macrophages of epithelioid type often in dense irregular clusters and multinucleated giant cells and depending on causative agent in a background of lymphocytes and plasma cells or necrosis with granulocytes (Figs. 1 and 2). Necrosis is common in infections by *Mycobacteria*, *Bartonella*, and fungi. Presence of eosinophil granulocytes is common in fungus infection. In the absence of necrosis, it is more suggestive of sarcoidosis.



Granulomatous Lymphadenitis, Cytological Findings, Fig. 1 Granulomatous lymphadenitis (tuberculosis): a necrotic background with a complex of epitheloid cells with indistinct cytoplasmic borders. MGG



Granulomatous Lymphadenitis, Cytological Findings, Fig. 2 Granulomatous lymphadenitis (sarcoidosis): a large irregular cluster of epitheloid cells with elongated often bent nuclei and poorly delineated cytoplasm. Small mature lymphoid cells can also be seen. MGG

Molecular Features

PCR assay allows identification of *M. tuberculosis*, *Bartonella*, and fungi.

Differential Diagnosis

Fungus infections, Hodgkin's lymphoma, T-cell lymphoma.

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Granulomatous Thyroiditis, Cytological Findings

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Synonyms

De Quervain's thyroiditis; Subacute thyroiditis

Definition

Virus-related inflammation of the thyroid gland, usually following a virus infection of the upper respiratory tract. It is a self-limited inflammatory disease.

Clinical Features

- **Incidence**
  The incidence rate is about 2.8/3.6.
- **Age**
  Medium-aged people.
- **Sex**
  More often women, with a female–male ratio of 2–3.5.
- **Site**
  The lesion may be found anywhere in the thyroid gland.
- **Treatment**
  Treatment includes analgesics, since it is a really painful disease, as well as anti-inflammatory drugs. In case of transitional hypothyroidism, a hormonal substitutive treatment is also required during the 2–5-month period.
- **Outcome**
  Usually there is no adverse outcome, and euthyroidism is the rule with healing. Nevertheless, a definitive hypothyroidism may

occur. Pain and generalized symptoms may exist for weeks if patients are left without any treatment.

Macroscopy

These lesions are usually not observed since surgical treatment is not relevant. Anyway wide thyroid increase could be observed with edema. The gland is firm and homogeneous.

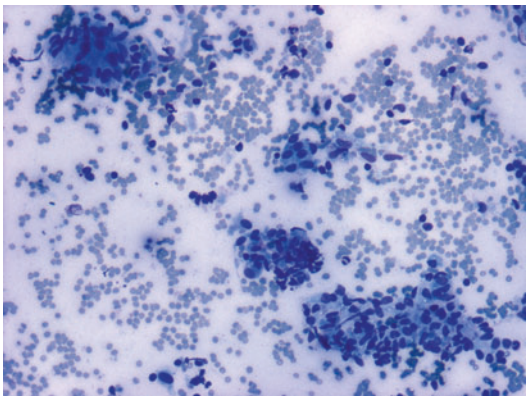
Microscopy

Fine-needle aspirations (FNA) are not necessary when the clinical symptoms are typical and when the gland is homogeneous on ultrasonography. Nevertheless, an FNA can be discussed later in order to eliminate a diagnosis of malignancy either when some nodules appear or when the gland is inhomogeneous and when the initial event is unknown or forgiven. The cellularity observed on the slides depends on the stage of the study. At the beginning, there are abundant neutrophils and eosinophils; the cytology is similar to an acute thyroiditis. Later the smears are typically hypocellular with presence of colloid, histiocytes ingesting droplets of colloid, and histiocytic giant cells as well as sheets of epithelioid cells (Figs. 1–3). There are few follicular cells and lymphocytes. The more typical features are represented by numerous granulomas with giant multinucleated cells. At the very late stage, granulomas are rare or absent and the FNA is then very often suboptimal in terms of cellularity.

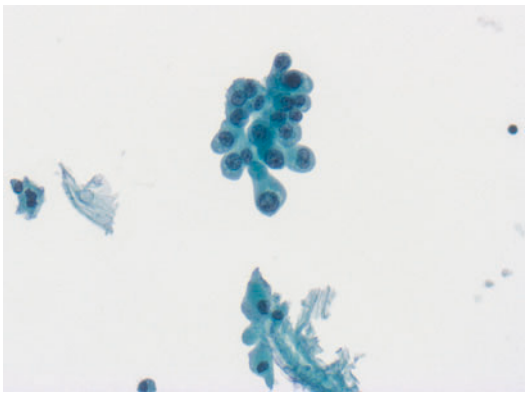
The features seen on liquid-based preparation are not very different from those observed on conventional smears. Nevertheless, lymphocytes as well as colloid may be rare or absent. Only the numerous giant cells, the epithelioid cells, and the hypocellularity may lead to the diagnosis.

Immunophenotype

Immunophenotyping is not necessary except for eventual virus typing.



Granulomatous Thyroiditis, Cytological Findings, Fig. 1 Few follicular cells with enlarged cytoplasm mixed with some epithelioid cells (notice the elongated nuclei) and with very few lymphocytes on the background (Conventional slide; MGG ×25)

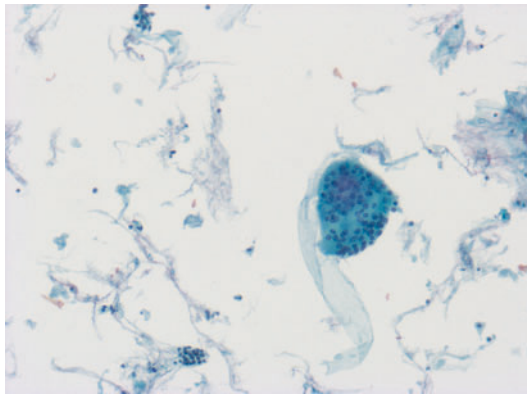


Granulomatous Thyroiditis, Cytological Findings, Fig. 2 Almost acellular slide with very few follicular cells and one group of epithelioid cells (LBC; Hologic®; Papanicolaou staining ×40)

Differential Diagnosis

Differential diagnoses include essentially other granulomatous diseases of the thyroid such as:

- Tuberculosis, with granulomatous cells and occasionally caseous necrosis. Thyroid is a quite rare localization.
- Sarcoidosis with non-caseating granulomas.
- Foreign body reaction with granulomatous reaction around catgut material after surgery.
- Acute thyroiditis at the beginning of the disease.



Granulomatous Thyroiditis, Cytological Findings, Fig. 3 Few dispersed follicular cells and one large giant histiocytic cell (LBC; Hologic®; Papanicolaou staining ×40)

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Granulosa Cell Tumor of Ovary, Cytological Findings

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Synonyms

Granulosa cell carcinoma; Granulosa cell tumor, malignant; Malignant granulosa cell tumor

Definition

Granulosa cell tumors are rare ovarian tumors divided into two subgroups: Adult granulosa cell tumor is more often found in postmenopausal woman and very rare variant, juvenile granulosa cell tumor is seen in the first three decades (Kavuri et al. 2010).

Macroscopy

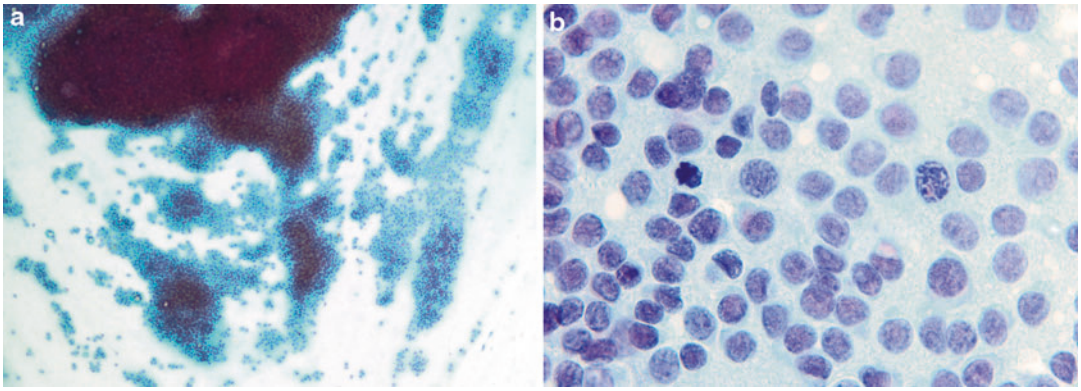
Macroscopically tumor is solid or cystic, yellow-white in color.

Microscopy

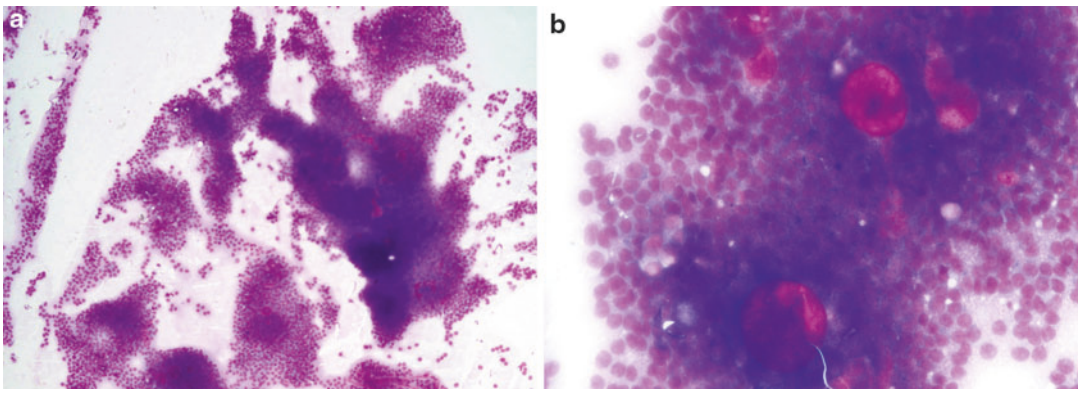
- **Imprints**
  Microscopically, slides are often hypercellular with numerous small and uniform granulosa cells arranged in large and small overlapping cell clusters, three-dimensional groups, loose cohesive sheets, a trabecular and follicular pattern, or without any architectural patterns. Numerous individual granulosa cells can be found (Fig. 1a) (Ivić et al. 1976; Kini 2011; Deb et al. 2011).

  Granulosa cells are homogenous in appearance, small to moderate in size and round to oval in shape. Cytoplasm is scant and delicate, with occasional fine vacuoles, often ill defined. The nuclei are centrally located, round to oval in shape with fine, evenly distributed chromatin. Hyperchromasia and prominent nucleoli can be found in both juvenile and adult type. Numerous naked nuclei are often found in the background. Nuclear grooves producing a coffee bean appearance can also be found in adult type. Mitotic activity can be increased in some cases (Fig. 1b) (Kavuri et al. 2010; Kini 2011; Manek and Mahovlic 2010).

  Small acinar-like structures of granulosa cells surrounding centrally placed amorphous eosinophilic fluid correspond to the Call-Exner bodies, can be found in some cases (Fig. 2a, b) (Kavuri et al. 2010; Ivić et al. 1976; Kini 2011; Deb et al. 2011; Manek and Mahovlic 2010).



Granulosa Cell Tumor of Ovary, Cytological Findings, Fig. 1 (**a**) Granulosa cell tumor. Highly cellular imprint with numerous small and uniform granulosa cells arranged in large and small overlapping cell clusters (Imprint, Papanicolaou ×100) (**b**) Granulosa cell tumor. Coarsely granular chromatin with parachromatin clearing, mitoses are seen (Imprint, Papanicolaou ×1,000)



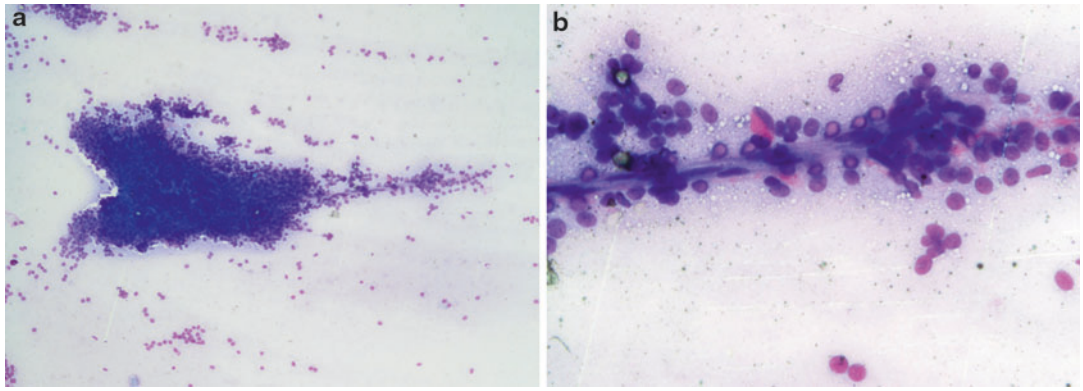
Granulosa Cell Tumor of Ovary, Cytological Findings, Fig. 2 (**a**) Granulosa cell tumor. Hypercellular imprint with multiple Call-Exner bodies (Imprint, MGG ×100) (**b**) Granulosa cell tumor. Uniform small cells can form acinar-like structures and Call-Exner bodies (Imprint, MGG ×400)

Blood vessels with prominent perivascular tumor cell growth and spindle shaped hyperchromatic stromal cells within cellular clusters can be present (Fig. 3a, b) (Kavuri et al. 2010; Kini 2011; Deb et al. 2011).

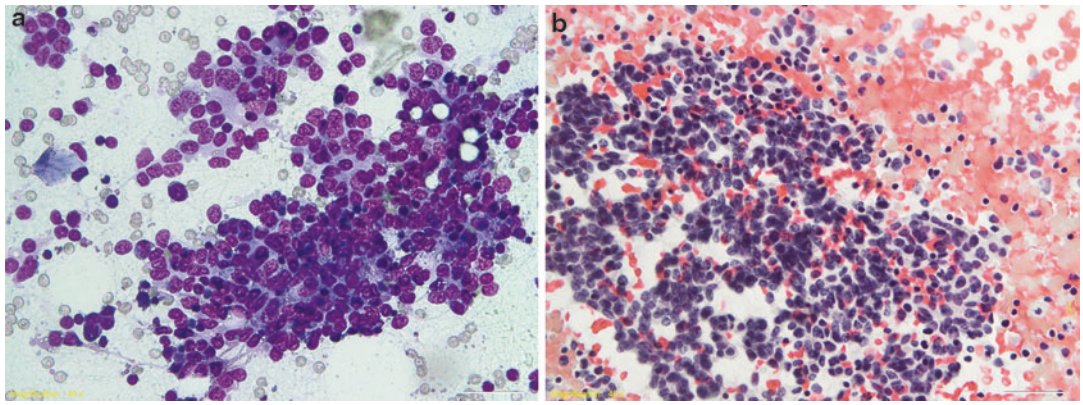
In the juvenile variant of granulosa cell tumor, cytological picture differs from its adult counterpart by the lack of prominent grooved nuclei, absence of Call-Exner bodies, and the presence of mucin and prominent lipid vacuoles in granulosa cells (Manek and Mahovlic 2010).

- **Fine Needle Aspiration**

  Macroscopically fluid of cystic tumors is usually clear to bloody (Kini 2011). Microscopically, cellularity is variable, background can be clean or bloody. Isolated or loosely cohesive groups and syncytial tissue fragments of granulosa cells can be found. Granulosa cells are small to moderate, usually somewhat larger than those seen in follicular cysts. The nuclei are round to oval in shape, sometimes with "coffee bean" pattern, with fine or coarsely granulated chromatin. Naked nuclei are often



Granulosa Cell Tumor of Ovary, Cytological Findings, Fig. 3 (**a**) Granulosa cell tumor. Spindle shaped hyperchromatic stromal cells within cellular clusters (Imprint, MGG ×100) (**b**) Granulosa cell tumor. Spindle shaped hyperchromatic stromal cells within cellular clusters (Imprint, MGG ×400)



Granulosa Cell Tumor of Ovary, Cytological Findings, Fig. 4 (**a**) Granulosa cell tumor. Lymph node metastasis (Fine needle aspiration, MGG ×400) (**b**) Granulosa cell tumor. Lymph node metastasis (Fine needle aspiration, Papanicolaou ×400)

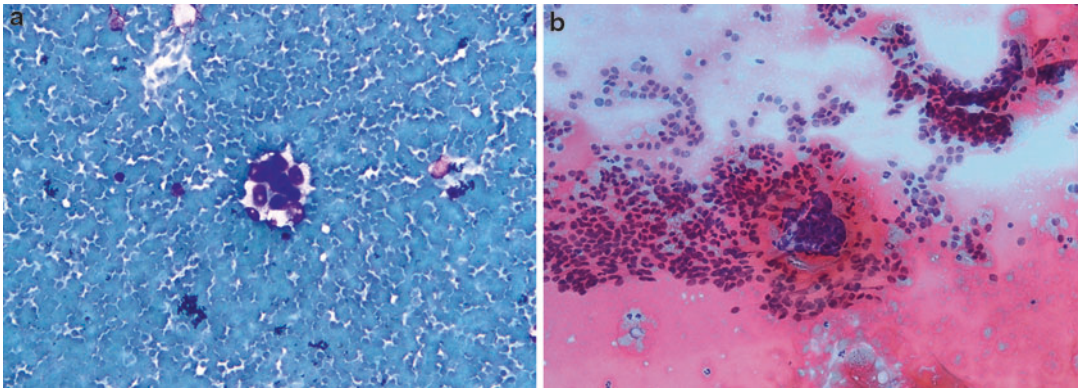
found in the background. The presence of Call-Exner bodies in some cases makes the diagnosis accurate (Kavuri et al. 2010; Kini 2011; Deb et al. 2011).

Cytological picture of granulosa cell tumor metastases to lymph node contains numerous granulosa cells arranged in sheets, a trabecular and follicular pattern, or without any architectural patterns (Fig. 4a, b).

- **Peritoneal Washing: Ascites**
  Fluids are often less cellular than imprints and cyst aspirate (Fig. 5a), but sometimes celullarity is abundant (Fig. 5b). Isolated small granulosa cells with scant or without cytoplasm can be difficult to recognize. Cell clusters when found are more compact than in imprints and cyst aspirates. Call-Exner bodies and stromal cells are usually not found (Kavuri et al. 2010; Kini 2011; Manek and Mahovlic 2010).

Immunophenotype

Immunocytochemistry: Granulosa cells are positive for calretinin, inhibin, vimentin, CD56 and CD99, and negative for CEA, EA, keratin, vimentin, and LCA. Positivity for EMA is variabile (Kini 2011; Manek and Mahovlic 2010).



Granulosa Cell Tumor of Ovary, Cytological Findings, Fig. 5 (**a**) Granulosa cell tumor. Small cluster of granulosa cells (Peritoneal washing, MGG ×400) (**b**) Granulosa cell tumor. Numerous granulosa cells (Peritoneal washing, Papanicolaou ×200)

Differential Diagnosis

Follicular cyst: Aspirates of functional cysts in reproductive-age patients can contain few to numerous dispersed granulosa cells or in syncytial tissue fragments. High cellularity, epithelial-like clusters, and cellular atypia in aspirates, as well as lack of luteinization may mimic granulosa cell tumor. The normal granulosa cells can mimic Call-Exner bodies (Kini 2011; Manek and Mahovlic 2010).

Luteinized follicular cysts: Large luteinized granulosa cells are performed with abundant granular or vacuolated cytoplasm. After IVF treatment, anisonucleosis and mitoses may be found (Kini 2011; Manek and Mahovlic 2010).

Corpus luteum and luteal cysts: Numerous luteinized granulose cells, hemosiderin-laden macrophages, fibrin, and a hemorrhagic background are found (Kini 2011; Manek and Mahovlic 2010).

Luteinized follicular cysts of pregnancy: Atypical luteinized granulosa cells with increased nuclear/cytoplasmic ratio, granular chromatin, and prominent nucleoli are performed (Kini 2011; Manek and Mahovlic 2010).

Adenocarcinoma (serous or poorly differentiated) can be diagnostic problem if the Call-Exner bodies or a coffee bean appearance of the nuclei is not present (Kini 2011; Manek and Mahovlic 2010).

Neuroendocrine tumors can contain large sheets of small cells with indistinct cytoplasm (Kini 2011; Manek and Mahovlic 2010).

Metastatic tumors with small cells can mimic granulosa cell tumour (Kini 2011; Manek and Mahovlic 2010).

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Gynecomastia, Cytological Findings

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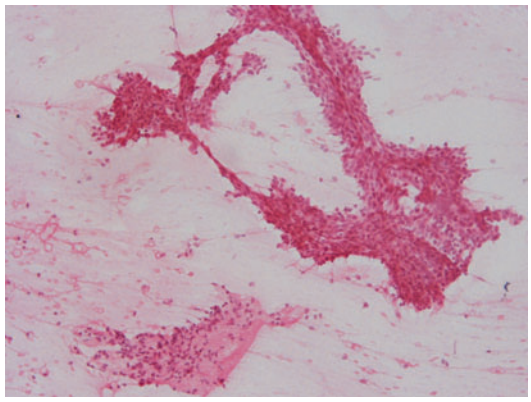
Synonyms

Gynecomastia

Definition

Gynecomastia (GM) is defined as a benign condition characterized by enlargement of the male breast, which is attributable to proliferation of the glandular tissue and local fat deposition (Fig. 1).



Gynecomastia, Cytological Findings, Fig. 1 Gynecomastia. Note the group of benign cells with naked nuclei at background and fragment of stroma (HE staining)

Clinical Features

- **Incidence/Age**
  It has been estimated that 30–60% of boys exhibit GM during adolescence and that at least one-third of the adult male population may be affected. It is more frequent in old men with a 65% prevalence. Increasing use of anabolic steroids and in environmental contamination with xenoestrogens or estrogen-like substances can stimulate glandular proliferation of the male breast.
- **Site**
  Male breast tissue proliferation may be unilateral or bilateral. Symptoms such as nipple discharge, ulceration, bleeding, and skin inversion may be indicative of underlying malignancy.
- **Treatment/Outcome**
  If the GM is slight, without noteworthy psychological repercussion, and the appropriate work-up does not reveal any underlying disease, reassurance and periodic follow-up visits (every 3–6 months) are recommended. If GM either persists or becomes more severe and is associated with pain, psychological distress, pharmacological, and surgical therapeutic options should be considered.

Macroscopy

It is sometimes difficult to differentiate between fatty tissue and breast tissue, especially in overweight individuals. Gynecomastia is clinically defined by the presence of a rubbery, firm mass extending concentrically from the nipples. This subareolar disk of glandular tissue has been described as feeling like a corded rope. In contrast, pseudogynecomastia is defined as the proliferation of soft subcutaneous fat that can give males the appearance of developing breasts.

Microscopy/Cytology

Cytologically, the aspirates are in general paucicellular with presence of large and small groups of

epithelial cells mixed with myoepithelial cells. These groups of cells are tightly cohesive, appearing as flat somewhat monolayered sheets, and can show mild to moderate atypia with cellular crowding with nuclear overlap and hyperchromasia. Presence of naked nuclei at background is frequent. Fatty-fibrous stroma can be present.

Differential Diagnosis

Diagnostic confusion with carcinoma may occur because of atypia and cellularity with loose groups and single cells; however, the mixed cell population, presence of bipolar naked nuclei, and lack of predominance of discohesive cells delineate GM from carcinoma of the male breast.

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H

Hashimoto Thyroiditis, Cytological Findings

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Synonyms

Lymphocytic thyroiditis

Definition

Autoimmune disease characterized by circulating antibodies against thyroglobulin and thyroperoxidase and usually increased prevalence of HLA DR3 or DR5.

Clinical Features

- **Incidence**
  It is the most frequent of the thyroiditis, and it affects about 1–2% of the population.
- **Age**
  Observed by middle-aged people, 30–60 year old and/or teenagers.
- **Sex**
  Very more frequent by women; ratio female: male is 5–10:1.
- **Site**
  The thyroid gland is totally involved; it appears usually enlarged and heterogeneous, sometimes with nodules, but some atrophic gland may also be found.
- **Treatment**
  Treatment is based on a substitutive hormonal treatment because hypothyroidism is usual.
- **Outcome**
  Usually, there is no adverse outcome. Nevertheless, it is often associated with other autoimmune diseases. Furthermore, the association with a carcinoma (papillary carcinoma) or even a lymphoma may occur (1%) justifying a close follow-up of these patients and fine-needle aspirations in case of nodules on ultrasonography.

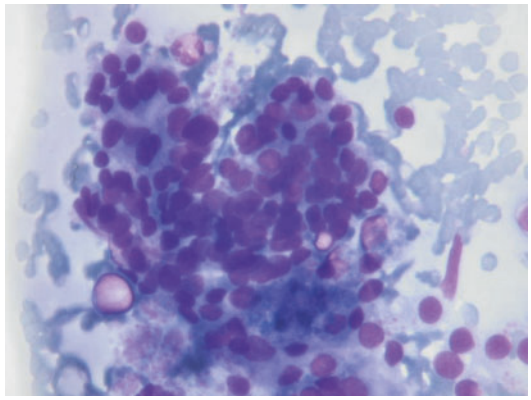
Macroscopy

These lesions are usually not observed since surgical treatment is not relevant. Anyway the gland should be enlarged or atrophic, more or less heterogeneous. True nodules may be observed, either benign or malignant.

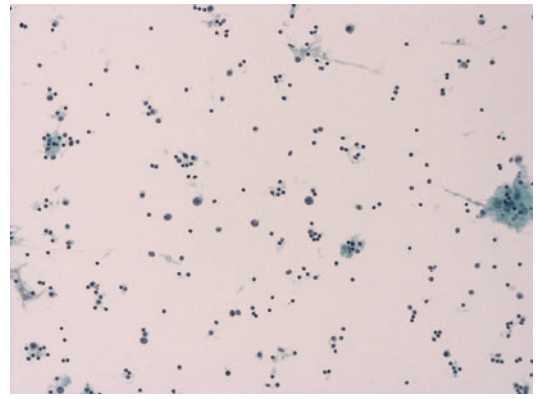
Microscopy

Fine-needle aspirations (FNA) are not necessary to do a diagnosis of Hashimoto disease. It is

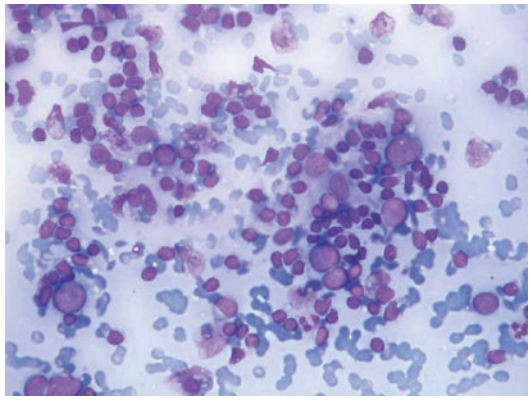
F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4



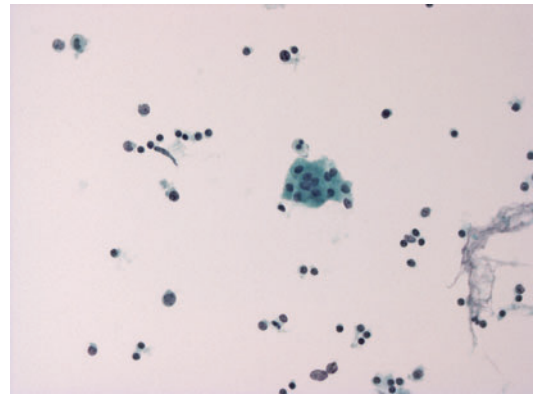
Hashimoto Thyroiditis, Cytological Findings, Fig. 1 Follicular cells with enlarged nuclei and slight overlapping surrounded by some lymphoid cells (conventional slide; MGG × 40)



Hashimoto Thyroiditis, Cytological Findings, Fig. 3 Isolated cells on the background with some monolayered sheets of follicular cells (LBC; Hologic®; Papanicolaou staining × 25)



Hashimoto Thyroiditis, Cytological Findings, Fig. 2 Polymorphic lymphoid cells, suggestive of thyroiditis (conventional slide; MGG × 25)



Hashimoto Thyroiditis, Cytological Findings, Fig. 4 Same case at higher magnification (LBC; Hologic®; Papanicolaou staining × 40)

a diagnosis based on clinical symptoms and biological results (serum antibody levels) Nevertheless, an FNA is required in case of nodules in order to detect an association with a carcinoma, mostly a papillary carcinoma or a lymphoma. Often the smears are characteristic. They are highly cellular including numerous follicular cells on a background of inflammatory, pleomorphic lymphocytic cells (Figs. 1 and 2). The epithelial component is represented by many follicular cells, which can display different grades of atypia, associated with Hürthle cells (Askanazy cells, oncocytic cells). The Hürthle cells are either single or in sheets; they have an enlarged basophilic or amphophilic cytoplasm, often granular due to their high number of mitochondria, and large, unique, or multiple, eccentric nuclei. Nuclear grooves may be observed but usually no pseudo-inclusions. Colloid is absent. Multinucleated giant cells may be present.

With liquid-based cytology, especially ThinPrep® (Hologic), the diagnosis is based on the same criteria; sometimes, the double component is easily recognizable (Figs. 3 and 4); in other cases, the diagnosis of Hashimoto thyroiditis is really challenging: The Hürthle cells are modified with pale, non-granular cytoplasm; the

nuclei may appear irregular with a smooth nuclear membrane; furthermore, the chronic inflammatory background is less abundant, sometimes really insufficient to assert the diagnosis.

Immunophenotype

Immunophenotyping is usually not necessary. CD3 and CD20 may be applied in cases of a monotonous lymphocytic background. Cytokeratin 19 and CD15, usually positive in cases of malignancy, may be strongly positive too in lymphocytic thyroiditis on the follicular cells; on the other hand, some other antibodies remain negative or weakly positive such as HBME1 and Galectine 3.

Molecular Features

Some polymorphism in immune regulatory genes and thyroid-specific genes could be implied in Hashimoto's thyroiditis as well as skewed-XCI could have a role in this autoimmune disease and might explain the female preponderance. BRAF mutations have not been observed in nodules in Hashimoto disease and could be a valuable test to avoid unnecessary surgical control in cases of atypia suggestive for a papillary carcinoma on cytology. In the case of suspected lymphoma, a new FNAC and flow cytometry study are indicated.

Differential Diagnosis

Pitfalls in Hashimoto´s disease include false-positives, either lymphoma or carcinoma, essentially papillary carcinoma, as well as false-negative when lymphocytic thyroiditis is associated with true carcinoma or lymphoma which is not recognized. In Hashimoto´s disease, marked atypia may appear in follicular cells, leading to a diagnosis of malignancy. In such cases, immunocytochemistry and molecular tests may be helpful as shown above; nevertheless, some cases cannot be solved by ancillary techniques and then surgical control cannot be avoided.

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Hepatoblastoma, Cytological Findings

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Synonyms

Primary embryonal malignant liver tumor

Definition

Hepatoblastoma is a rare malignant liver tumor of infancy and more rarely of adults composed of cells resembling fetal liver cells that may have a stromal component or an undifferentiated component.

Clinical Features

- **Incidence**
  It is the most frequent hepatic malignant tumor of infancy. The incidence is 0.9 per million children (Finegold et al. 2007). It is higher in premature and low-weight infants which probably accounts for the increase incidence in developed countries. Table 1 is a list of syndromes and conditions associated with hepatoblastomas.
- **Age**
  Sixty percent appear under the age of 2 years and 90% before age 5 years (Finegold et al. 2007). It comprises around 4% of tumors at the neonatal period (Lakhoo and Sowerbutts 2010). Although rare, it may appear in the adult.
- **Sex**
  Male:female ratio is 2:1 (Ferrel 2009).
- **Site**
  Ninety percent occur in non-cirrhotic liver.
- **Symptoms**
  Abdominal mass due to hepatomegaly and a liver mass detected at ultrasonography are the two main alert features. Failure to thrive, weight loss, vomiting, diarrhea, and jaundice may occur. More rarely, there may be pubertal precocity and virilization related to hormone production.
- **Treatment**
  Treatment options include surgery alone or in combination with chemotherapy, chemoembolization, and liver transplant. The first aim is to resect the lesions, but unfortunately, it only happens in 67% of the cases.
- **Outcome**
  Outcome depends on staging (Table 2). Stage I well-differentiated fetal hepatoblastoma can be treated with surgery alone. On the other hand, small cell type is typically found in infants younger than 1 year; it has a poor prognosis, with poor response to current therapy (Finegold et al. 2007).

  There is no prognostic significance to the presence of mixed histologic features.

Hepatoblastoma, Cytological Findings, Table 1 Clinical syndromes, congenital malformations, and other conditions associated with hepatoblastoma (Reproduced from Finegold et al. (2007)

Congenital malformations
Absence of left adrenal gland
Bilateral talipes
Duplicated ureters
Dysplasia of ear lobes
Cleft palate
Fetal hydrops
Hemihypertrophy
Heterotopic lung tissue
Horseshoe kidney
Inguinal hernia
Intrathoracic kidney
Macroglossia
Meckel diverticulum
Persistent ductus arteriosus
Renal dysplasia
Right-sided diaphragmatic hernia
Single coronary artery
Umbilical hernia
Syndromes
Beckwith-Wiedemann syndrome
Beckwith-Wiedemann syndrome with opsoclonus, myoclonus
Budd-Chiari syndrome
Familial adenomatous polyposis syndrome
Li-Fraumeni cancer syndrome
Polyposis coli families
Schinzel-Giedion syndrome
Trisomy 18
Metabolic/pathophysiologic abnormalities
Cystathioninuria
Glycogen storage disease types Ia, III, and IV
Hypoglycemia
Heterozygous 1-antitrypsin deficiency
Isosexual precocity
Prematurity
Total parenteral nutrition
Very low birth weight
Environmental/other
Alcohol embryopathy
Human immunodeficiency virus or hepatitis B virus infection
Maternal clomiphene citrate or Pergonal
Oral contraceptive, mother
Oral contraceptive, patient
Osteoporosis
Synchronous Wilms tumor

Hepatoblastoma, Cytological Findings, Table 2 The Children's Oncology Group staging system recommended for hepatoblastomas (Reproduced from Finegold et al. (2007))

Stage I (favorable histologic type) tumors are completely resected and have typical histologic features of a purely fetal well-differentiated histologic pattern (minimal mitotic index of ≤2 mitoses per 10 high power [×40 objective] fields)
Stage I (other histologic type) tumors are completely resected, with a histologic picture other than purely fetal, well-differentiated pattern
Stage II tumors are grossly resected with evidence of microscopic residual tumor. Such tumors are rare, and patients with this stage have not fared differently from those with stage I tumors in previous protocols. Resected tumors with preoperative (intraoperative) rupture are classified stage II
Stage III (unresectable) tumors are those that are considered by the attending surgeon not to be resectable without undue risk to the patient. These include partially resected tumors with measurable tumor left behind. They do not include grossly resected tumors with microscopic disease at the margins or resected tumors with preoperative/intraoperative rupture. Lymph node involvement is considered stage III disease and may require evaluation with second laparotomy after initial 4 courses of chemotherapy
Stage IV tumors are those that present with measurable metastatic disease to the lungs or other organs

Hepatoblastoma, Cytological Findings, Table 3 Classification of hepatoblastoma (Reproduced from Finegold et al. (2007))

Major categories
Epithelial
Fetal, well differentiated (mitotically inactive with minimal mitotic rate of ≤2 mitoses per 10, ×40 objective fields)
Fetal, mitotically active (>2 mitoses per 10, ×40 objective fields)
Embryonal
Macrotrabecular
Small cell, undifferentiated
Rhabdoid
Mixed stroma having osteoid features; rarely striated muscle, cartilage, or minor components as follows:
Cholangioblastic (ductal)
Intestinal glandular epithelium (teratoid)
Neuroid-melanocytic (teratoid)
Rhabdomyoblastic
Chondroid
Blastemal

Macroscopy

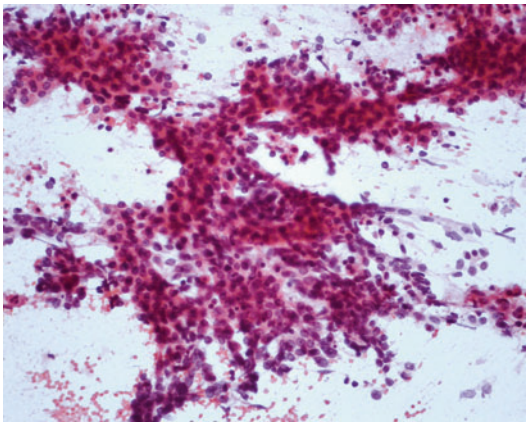
Normally, it is a multinodular mass, frequently hemorrhagic, and necrotic. It is characteristic that different nodules have diverse types of tumor tissue, as described below.

Microscopy

Classification of hepatoblastoma is present in Table 3. Although less frequent, morphological entities can present different cell types as depicted, three main types of cells are fetal, embryonal, and small cell undifferentiated.

Smears show predominantly epithelial cellularity and scant mesenchymal component or heterologous elements (Fig. 1).

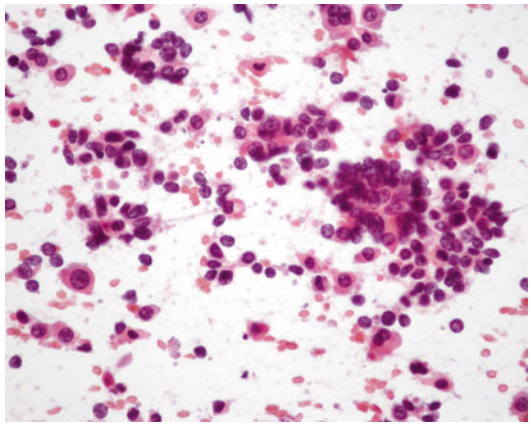
Fetal cell type is identical to a small hepatocyte. Nucleus are smaller, but N:C ratio is higher.



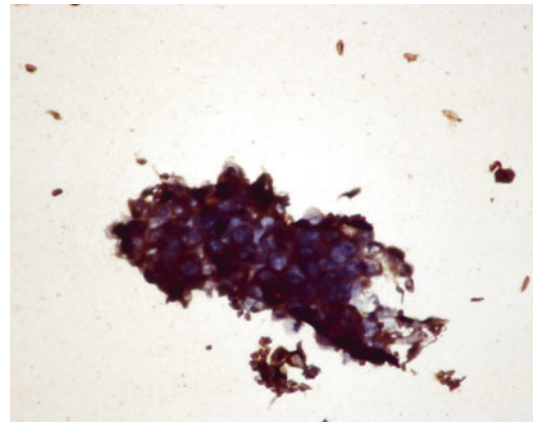
Hepatoblastoma, Cytological Findings, Fig. 1 Smears with predominant epithelial cells (H&E) (Author)

Cytoplasm may have lipidic or glycogen vacuoles (Pitman 2010).

Embryonal type cells are smaller and less differentiated. They can form rosettes and trabeculae (Fig. 2). Small cell types (anaplastic) are identical to other small round-blue cells from Wilms' tumor or neuroblastoma.



Hepatoblastoma, Cytological Findings, Fig. 2 Cells look like a small hepatocyte. They may have lipid droplets (center of field). On the *right* a group of cells form a rosette (H&E) (Author)



Hepatoblastoma, Cytological Findings, Fig. 3 Alpha-FP positivity in tumor cells: immunohistochemistry (Author)

Mesenchymal cells are spindled, and heterologous elements like osteoid, cartilage, skeletal muscle may appear.

Extramedullary hematopoiesis is particularly associated with fetal type morphology.

Immunophenotype

HepPar1-1 and α-FP are positive in epithelial cells (Fig. 3). They also express high molecular weight cytokeratins.

Genetic Features

The most common karyotypic changes are extra copies of entire chromosomes (trisomies), especially chromosome 2 and 20, sometimes in conjunction with other complex structural changes and often in association with double-minute chromosomes.

Differential Diagnosis

Primary diagnosis by cytology (fine-needle aspiration) may be misleading because of difficulties in distinguishing from well-differentiated hepatocellular malignancy, regenerative changes, and benign proliferations, and because of the variability of histologic features in hepatoblastoma.

Clinical-morphological correlation is of upmost importance along with ancillary tests.

Alpha-fetoprotein is of little use in the differential diagnosis. High molecular weight cytokeratins will mark epithelial cells from hepatoblastoma and cells from hepatocellular carcinoma will not. HepPar1 is useful in undifferentiated cell type of tumors composed of small round-blue cells. Hepatoblastoma cells are positive with this antibody.

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Hepatocellular Carcinoma, Cytological Findings

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Synonyms

Malignant hepatoma

Definition

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver.

Clinical Features

Hepatocellular carcinoma is the third most common cause of cancer-related death worldwide, and it still is rising in the developing and developed countries due to prevalence of two of the major risk factors, hepatitis B virus and hepatitis C virus (Shariff et al. 2009). Other risk factors include obesity, diabetes, and related non-alcoholic fatty liver disease. Cirrhosis from causes other than alcohol carries a higher risk too like hemochromatosis and alpha1-anti-trypsin deficiency.

The American Association for the Study of Liver Diseases guidelines recommend routine cancer surveillance among cirrhotic HBV carriers, non-cirrhotic HBV carriers of Asian ethnicity (males over the age of 40 years and females over the age of 50 years), and Africans over the age of 20 years. HCC surveillance is also recommended among chronic HBV patients over the age of 40 years if they have persistent inflammatory activity on biopsy, elevated liver enzyme levels, and/or HBV DNA levels above 2,000 IU/mL and also with any form of cirrhosis. Eighty-five percent occur in a setting of cirrhosis (Bruix and Sherman 2010).

For screening, they recommend ultrasonography at 6-month intervals, for patients at high risk of developing HCC.

Symptoms may be vague and include abdominal pain, weight loss, jaundice, ascites, malaise, and fever, but may be asymptomatic. Rupture with hemorrhage may be a complication.

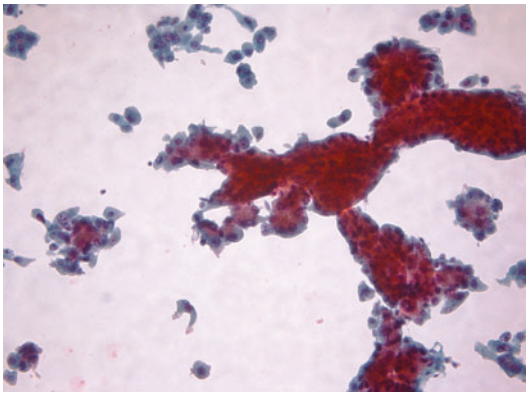
In two thirds of the cases, serum alpha-fetoprotein is elevated above 1,000 ng/mL (Wong and Frenette 2011).

- **Age and Sex**
  In the United States, age of incidence is around 60 years, but in Africa, it is 30 years. It is more frequent in men than in women (3:1).
- **Treatment**
  The Barcelona Clinic Liver Cancer staging system model is currently the most comprehensive and widely accepted staging system for HCC. This model incorporates variables

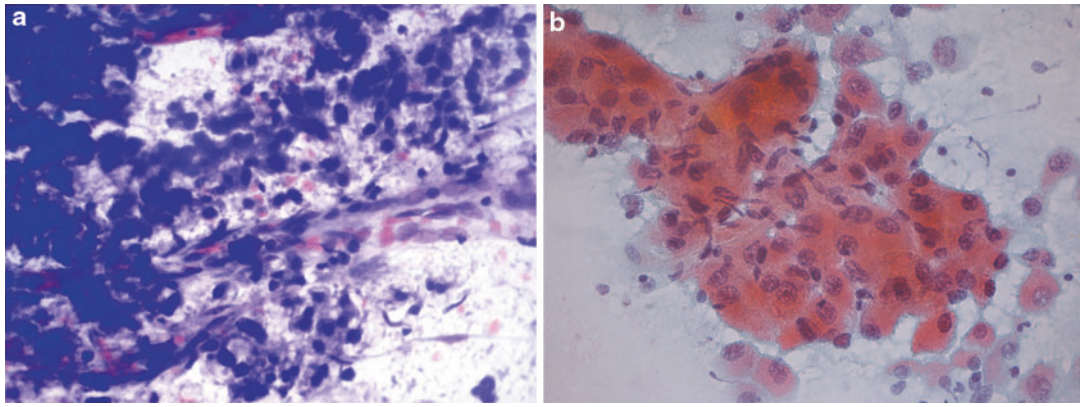
Hepatocellular Carcinoma, Cytological Findings, Table 1 *Histological Patterns of Hepatocellular Carcinoma* (*HCC*) (may appear alone or combined)

Trabecular
Acinar
Solid
Cirrhotic
Clear cell
Patterns with particular clinicopathological aspects
Fibrolamellar
Combined (Cholangiocarcinoma and HCC)

Author from: Bosman et al. (2010)



Hepatocellular Carcinoma, Cytological Findings, Fig. 1 Cells arranged in nests and trabecula. Nucleus from wrapping endothelial cells can be seen along the large trabecula (Pap stain)



Hepatocellular Carcinoma, Cytological Findings, Fig. 2 Discohesive sheets of cells with transgressing vessels and endothelial cells: (**a**) MGG stain; (**b**) Pap stain

reflecting tumor stage, liver function status, and cancer-related symptoms (A table with Barcelona Clinic Liver Cancer staging classification and treatment schedule can be seen at Llovet et al. 2008).

Treatment options are based on a multidisciplinary approach, and consist of combinations of locoregional therapy, surgical resection, orthotopic liver transplantation, and systemic chemotherapy (Wong and Frenette 2011).

- **Outcome**
  The best rates of cure are achieved with surgical resection or orthotopic liver transplantation in well-selected patients, and the outcome is dictated by the adequacy of resection balanced with the preservation of liver function.

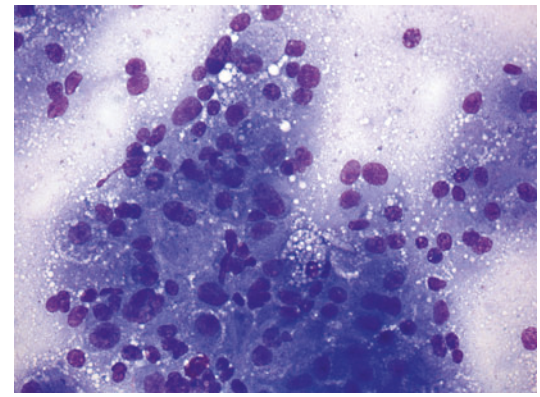
Macroscopy

HCC may present as a solitary hepatic nodule, but more frequently, it is multinodular. Sometimes, they have a fibrous capsule. Portal vein invasion may be seen.

Microscopy

Several histological patterns (Ferrel 2009; Bosman et al. 2010) are described and some are reproduced on cytology (Table 1).

Three basic patterns of cells are described:

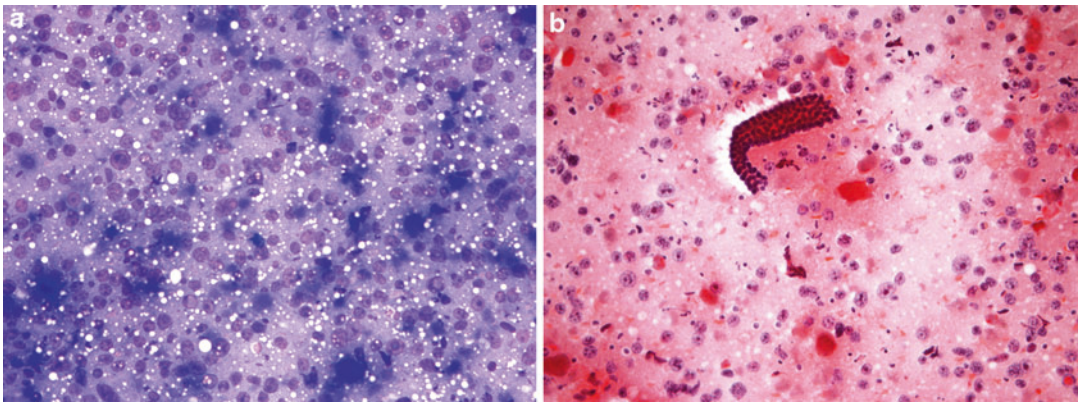


Hepatocellular Carcinoma, Cytological Findings, Fig. 3 Dispersed clusters without vascular pattern (MGG stain)

1. Cohesive, nested, and trabecular with wrapping endothelial cells (Fig. 1)
2. Loosely cohesive sheets with transgressing vessels or endothelial cells (Fig. 2)
3. Small clusters, dispersed, without vascular pattern (Fig. 3)

On cell-blocks or tissue fragments, morphology of these patterns described can be appreciated as well and, if needed, apply ancillary tests like immunocytochemistry (Cell-blocks).

Smears with monotonous cytology, elevated N:C ratio, macronucleoli, and intracytoplasmic hyaline globules (Pitman 2010) will aid to make a malignant diagnosis (Fig. 4).



Hepatocellular Carcinoma, Cytological Findings, Fig. 4 (**a**) Dispersed cells with elevated N:C ratio and macronucleoli (MGG stain); (**b**)intracytoplasmic hyaline globules; a benign sheet of ductal cells can be seen at the center of the field (Pap stain)

Immunophenotype

Hep-Par, pCEA, or CD10 stain up the hepatocytes in benign, dysplastic, and malignant conditions; hence, they are not very contributory in solving diagnostic problems, while AFP has a low sensitivity of about 50% (Cheuk-Lam Lo and Oi-Lin Ng 2011).

A panel of immunohistochemical markers including glypican-3, heat shock protein-70, and glutamine synthetase has been studied to be used in the diagnosis of small, well-differentiated hepatocellular tumors and particularly of HCC.

Also, the role of tissue biomarkers for prognostication in HCC and subclassifying HCC based on tumor cell origin have been studied (Cheuk-Lam Lo and Oi-Lin Ng 2011) (CD10, CD34).

Differential Diagnosis

Differential diagnosis of HCC is with benign nodules like adenoma and focal nodular hyperplasia, especially well-differentiated HCC (grade 1), on the one hand, and on the other hand, with metastatic tumors mainly adenocarcinoma and cholangiocarcinoma, when the acinar pattern is dominant. Besides morphology, immunocytochemistry can be useful (Cheuk-Lam Lo and Oi-Lin Ng 2011).

Alpha-fetoprotein will be positive in only 50% of HCC, but other useful markers include CEAp, Glypican 3, CK8/18, CK7/19, and Melan A, Chromogranin, among others against specific tumor types (▶ Liver Metastasis, Cytological Findings).

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Herpes Virus, Cytological Findings

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Synonyms

Cold sores (type 1); Genital blisters; Genital herpes infection; Genital herpes; Genital sores; Herpes simplex; herpes simplex virus; Herpesviridae; HSV-1; HSV-2; Human herpes 1 & 2; Sexually Transmitted Disease; Sexually transmitted infection; STD; STDs; STI; STIs

Definition

Herpes simplex (Greek: ἕρπης – herpes, lit. "creeping") is a viral disease caused by herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (http://en.wikipedia.org/wiki/Herpes_simplex). Genital herpes is mainly caused by HSV-2 and occasionally by HSV-1. The HSV is transmitted during close personal contact through the exchange of saliva, semen, cervical fluid, or vesicle fluid from active lesions (http://dermatology.about.com/cs/genitalherpes/a/genherp_women.htm).

Clinical Features

- **Incidence**
  According to Xu F et al., in the USA, 17.2% of the population is HSV-2 sero-positive and only 14.5% of the sero-positive population was aware that they are infected (Xu et al. 2006).
- **Age**
  Genital herpes (type 2) generally infects individuals over the age of 12; however, oral herpes (type 1) can be sero-positive in much younger age groups.
- **Sex**
  Women are approximately four times more likely to acquire a HSV 2 infection than men (http://dermatology.about.com/cs/genitalherpes/a/genherp_women.htm).
- **Symptoms**
  Genital herpes leads to blisters or small ulcers (open sores) on and around the genitalia in both men and women. The pustular lesions or small ulcers may coalesce to form larger ulcers and may persist from 4 to 15 days until crusting and healing occurs.

  Primary genital herpes is associated with HSV cervicitis in 90% of patients (Corey et al. 1983) and may be accompanied by systemic symptoms. Although the disease is self-limiting, recurrent episodes occur in some women. HSV can involve only cervix without involvement of external genitalia and such patients may be asymptomatic (Levine and Gray 2010). The squamous as well as endocervical epithelium can be equally affected in the cervix (Titmuss and Adams 2007). The infection is especially significant in pregnant women with genital herpes, because the virus can be transmitted at the time of birth and can cause severe damage to the newborn infant (Titmuss and Adams 2007).

- **Treatment**
  Prevention is better than cure and barrier methods of contraception can reduce the transmission risk. There is no method to completely eliminate herpes virus from the body, but antiviral medications can reduce the severity, frequency, and duration of outbreaks. Acyclovir is the recommended antiviral for herpes suppressive therapy.
- **Outcome**
  Following active infection, herpes viruses establish a latent infection in sensory and autonomic ganglia of the nervous system and many HSV-infected people have recurrence within the first year of infection. Subsequent outbreaks may be periodic or episodic, occurring on an average four to five times a year when not using antiviral therapy.
- **Relation with cervical cancer**
  Although HSV-2 infection may act in conjunction with human papilloma virus (HPV) infection to increase the risk of invasive cervical cancer, the effect of HSV-2 infection on invasive cervical cancer risk is modest compared with the strong effect of HPV infection on invasive cervical cancer risk (Smith et al. 2002).

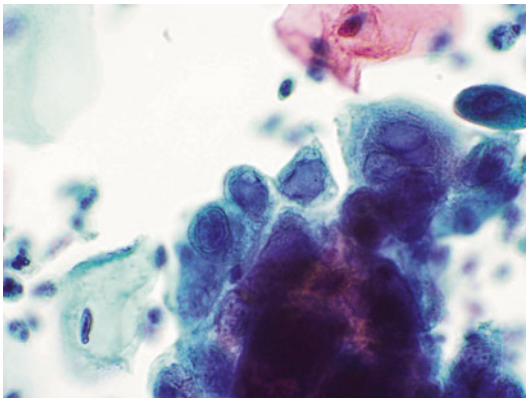
Cytomorphology

Nuclear changes are characterized by (Titmuss and Adams 2007) (Fig. 1)

- Multinucleation, nuclear molding
- Margination of nuclear chromatin
- Loss of nuclear chromatin structure resulting in ground-glass appearance
- Eosinophilic or amphophilic intranuclear inclusion bodies (Cowdry's bodies) occupying the whole nucleus and surrounded by a clear intranuclear halo

Cytoplasmic characteristics include (Titmuss and Adams 2007)

- Abundant basophilic vacuolated cell cytoplasm



Herpes Virus, Cytological Findings, Fig. 1 A microphotograph showing nuclear features of herpes virus infection with multinucleation, nuclear molding, margination of nuclear chromatin with ground-glass appearance of nuclear chromatin and intranuclear Cowdry's bodies surrounded by a clear halo (Pap ×100X)

- Indistinct cellular outline with cellular degeneration

Differential Diagnosis

Multinucleation in endocervical cells
Varying degree of squamous dyskaryosis
Glandular abnormality

HSV Detection Tests

Tests for HSV are most often performed only for sores in the genital region (http://www.webmd.com/genital-herpes/herpes-tests).

- Herpes viral culture
- Herpes virus antigen detection test (http://www.webmd.com/genital-herpes/herpes-tests)
- Polymerase chain reaction (PCR) test (http://www.webmd.com/genital-herpes/herpes-tests)
- Antibody tests
- Immunoperoxidase staining is very specific but not as sensitive as routine Papanicolaou-stained smears in the detection of HSV (Wong et al. 1985).

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High-Grade Urothelial Carcinoma, Cytological Findings

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Synonyms

Transitional cell carcinoma (term not recommended)

Definition

Historically, urothelial tumors were categorized into the three histological groups, grade 1, grade 2, and grade 3, that largely matched with the three-tiered cytological grading systems commonly and also proposed by L. Koss. In 1998, the World Health Organization and International Society of Urologic Pathologists (WHO/ISUP) established a new histological classification system for urothelial neoplasm that was incorporated in the 2004 WHO "Blue Book." As opposed to the previous WHO classification from 1973, the three-tiered grading system was simplified to a low-grade and high-grade group, with the low-grade group almost exclusively being diagnosed in noninvasive neoplasia including pTa tumors and dysplasia (low-grade intraurothelial neoplasia). As opposed to the previous G3, the threshold between low-grade and high-grade cuts right through the former G2 category. These lowered criteria might affect the accuracy of cytology for the detection of high-grade lesions, because most of the former WHO G2 lesions are now placed into the new high-grade urothelial carcinoma. Nevertheless, since high-grade neoplasia including carcinoma in situ primarily relies on cytological and nuclear features, detection of high-grade urothelial lesions in voided urines and washings of the bladder and the upper urinary tract remain the major strength and primary scope of urinary cytology. The cytological detection of high-grade urothelial carcinoma (HGUC) is emphasized in the forthcoming new "Paris system of reporting urinary cytology" by the cytological categories of "HGUC" and "atypical urothelial cells, suggestive of HGUC" (AUC-H) to be separated from less important other lesions. This is particularly important in voided urines from patients with hematuria or a history of UC, where cytology can detect high-grade UC from the whole urothelial tract including the renal pelvis, ureter, bladder, and urethra. In bladder washings, cytology mainly serves the detection of carcinoma in situ that is often difficult to discern from inflammatory lesions at cystoscopy.

Clinical Features

- **Incidence**
  Bladder cancer is the most common malignancy in the urinary tract and the 7th most

common cancer in men and the 17th in women. HGUC makes approximately 30% of all urothelial carcinomas.

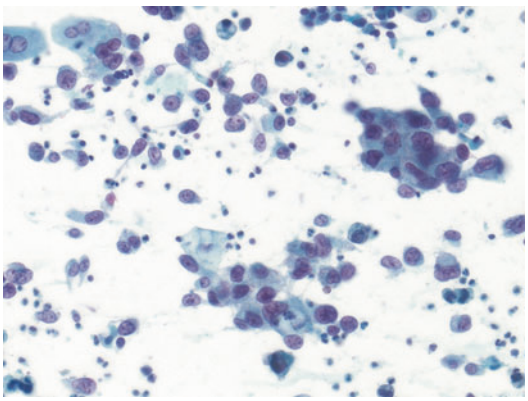
- **Age**
  Urothelial tumors are typically a disease of elderly people. The median ages of patients at the time of the initial diagnosis are 69 years in men and 71 years in women.
- **Sex**
  More common in male than in female (the ratio is about 3.5:1). Smoking increases the risk of bladder cancer by a factor of 2–6.
- **Site**
  Urothelial carcinomas are often multifocal due to a cancer field effect of the whole bladder and upper urinary tract or due to intraepithelial spreading of tumor cells. Approximately 5–10% of all recurrences occur in the upper urinary tract.
- **Treatment**
  Non-muscle-invasive high-risk tumors are treated with intravesical treatment with Bacille Calmette-Guérin (BCG) after resection. This BCG treatment leads to an immunological reaction against tumor cells and reduces recurrence and progression to muscle-invasive disease. Radical cystoprostatectomy or partial cystectomy is the treatment of choice in patients with muscle-invasive bladder cancer.
- **Outcome**
  The prognosis largely depends on the pathological stage and grade of the disease. About half of the patients with carcinoma in situ are successfully treated with BCG instillation. Depending on the stage (pT2 vs. pT3) or nodal status (pN0 vs. pN1), 40–70% of patients with muscle-invasive urothelial carcinoma succumb to the disease despite radical surgery.

Macroscopy

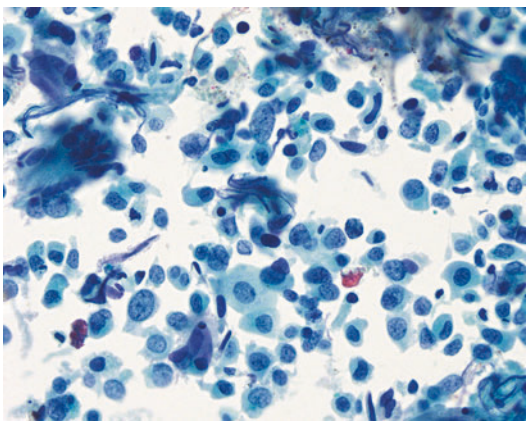
The procedures for obtaining and processing voided urines and bladder washings are described elsewhere in detail.

Microscopy

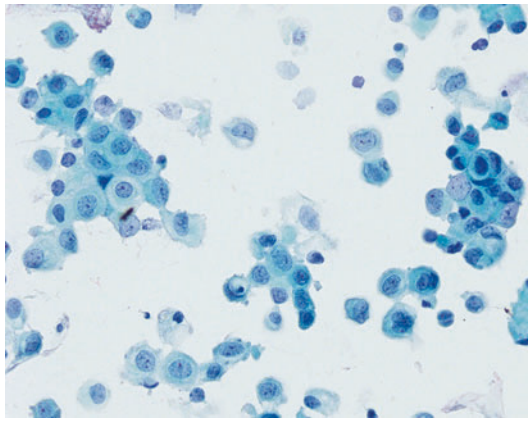
Detection of high-grade UC including carcinoma in situ is the major strength of cytology. The cytological diagnosis of high-grade UC and carcinoma in situ is straightforward. Representative examples of cells from HGUC in bladder washings are shown in Figs. 1, 2, and 3. The tumors cells are often discohesive and catch the eye by their dark, hyperchromatic nuclei, which show



High-Grade Urothelial Carcinoma, Cytological Findings, Fig. 1 High-grade urothelial carcinoma (G3), numerous discohesive cells with eccentric, polymorphic nuclei, irregular chromatin structure, and prominent nucleoli (bladder washing, Papanicolaou ×400)



High-Grade Urothelial Carcinoma, Cytological Findings, Fig. 2 High-grade urothelial carcinoma (G2), numerous malignant urothelial cells with eccentric, enlarged, and polymorphic nuclei; irregularly structured chromatin; and prominent nucleoli (bladder washing, Papanicolaou ×400). Histology revealed carcinoma in situ



High-Grade Urothelial Carcinoma, Cytological Findings, Fig. 3 High-grade urothelial carcinoma (G2), numerous discohesive cells with eccentric, polymorphic nuclei; irregular chromatin structure; and prominent nucleoli (bladder washing, Papanicolaou ×400). Histology revealed pTa high-grade urothelial carcinoma

high variability in size and shape, and an irregular and thickened nuclear membrane. The chromatin is irregularly structured and usually coarsely granular with or without prominent nucleoli. In some instances, the nuclei have an open chromatin, but there are still some coarse granula and a thickened, irregular nuclear membrane. The nuclei are eccentric and the nuclear-cytoplasmic ratio is high. As a rule of thumb, an unequivocal high-grade UC can be diagnosed without much thinking within 5 s. As part of a biological continuum, the former grade 2 encompasses both the lower end of high-grade UC and the higher end of low-grade UC. The former G2 cells are less obviously malignant than the G3 cells, but should be assigned to the high-grade category if they show eccentric, polymorphic, dark, and hyperchromatic nuclei with a coarse chromatin and a clearly irregular contour of the nuclear membrane. Presence of HGUC cells with tumor diathesis consisting of blood and cellular debris in the background hints towards invasive UC. A clear background, instead, is a typical finding in carcinoma in situ. However, the quality of background is an uncertain diagnostic feature.

There are rare variants of UC defined by special growth patterns or features including the plasmacytoid, micropapillary, and nested variants, urothelial carcinoma with small tubules, microcystic urothelial carcinoma, and others. Although some of these can be recognized or at least suspected by cytology, the main task remains the diagnosis of high-grade urothelial carcinoma by general criteria irrespective of variants.

Immunophenotype

Urothelial cells are diffusely positive for cytokeratin 7, p63, and GATA-3. Cytokeratin 20, which mainly stains normal umbrella cells in the benign urothelium, becomes often diffusely positive in urothelial carcinoma. These markers can be used to differentiate UC from infiltration or metastasis of an extra-vesical carcinoma in cases in which a distinction based on morphology alone is difficult. Most of the high-grade UC (at least the former G3) including carcinoma in situ are diffusely positive for p53 due to missense p53 mutation and abnormal accumulation of inactive p53. Some of the tumors may also reveal a complete loss of p53 expression caused by a nonsense p53 mutation.

Molecular Features

Virtually all high-grade urothelial carcinomas are chromosomally instable and aneuploid, which goes in parallel with the high degree of nuclear atypia. Not surprisingly, the UroVysion® multitarget fluorescence in situ hybridization (FISH) assay (Abbott Molecular Inc., Des Plaines, IL) for detecting polysomies of chromosomes 3, 7, and 17 and deletion of 9p21 is invariably positive in high-grade UC. Given the high specificity of cytology for the diagnosis of high-grade urothelial neoplasia, molecular testing is generally not needed. There are currently no established predictive markers to guide personalized treatments in bladder cancer.

Differential Diagnosis

Pronounced reactive changes after BCG treatment are notoriously difficult and can be misinterpreted as persistent or recurrent carcinoma in situ depending on the experience of the cytologist. The nuclear details and the nuclear-cytoplasmic ratio are critical for a correct diagnosis. Decoy cells in patients with activated polyoma virus due to immunosuppression (e.g., after kidney transplantation) can masquerade as high-grade UC cells and mislead inexperienced cytologists to a false-positive diagnosis. Poorly differentiated urothelial carcinoma might be mimicked by locally advanced, poorly differentiated adenocarcinomas of the prostate with a solid or diffuse growth pattern. Besides clinical history and information on the serum PSA value, a limited immunocytochemical marker panel including cytokeratin 7 and GATA-3 (consistently positive in urothelial carcinoma but negative in prostate cancer) and PSA and ERG (negative in urothelial carcinoma but positive in a large fraction of prostatic adenocarcinomas) can reliably solve this question. Benign seminal vesicle cells may rarely be encountered in voided urines and raise concerns due to remarkable cytological atypia, but the characteristic intracytoplasmic golden-yellow pigment (lipofuscin) reveals their true nature.

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Hodgkin Lymphoma, Cytological Findings

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Synonyms

Hodgkin's disease; Lymphogranulomatosis

Variants

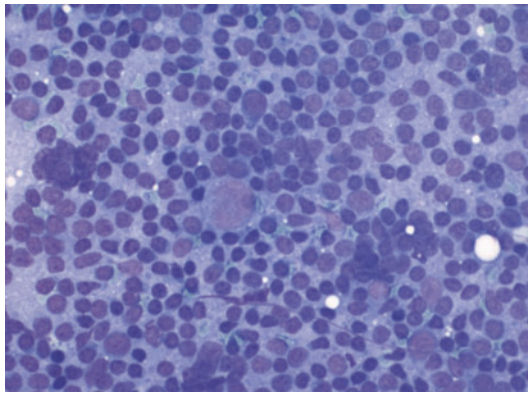
1. Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)
2. Classical Hodgkin lymphoma (CHL)

Definition

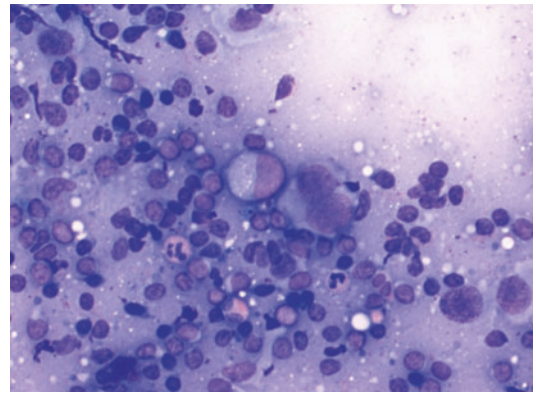
Lymph node disease with few large mono- and binucleated tumor cell mixed with a rich number of non-neoplastic lymphocytes and various inflammatory cells

Clinical Features

- **Incidence**
  NLPHL: 0.2/100,000
  CHL: 4/100,000
- **Age**
  NLPHL: middle aged
  CHL: young adults and elderly
- **Sex**
  NLPHL: male predominance



Hodgkin Lymphoma, Cytological Findings, Fig. 1 Hodgkin's lymphoma – nodular lymphocytic predominant: small mature B- and T-cells and few centroblasts. One large neoplastic cell with lobated nucleus "popcorn cell" (*center*). MGG



Hodgkin Lymphoma, Cytological Findings, Fig. 2 Hodgkin's lymphoma – classical: small mature lymphocytes and eosinophil granulocytes together with large mononuclear Hodgkin cells and one binucleated Reed-Sternberg cell (*center*). MGG

CHL: somewhat more common in males than in females.

- **Site**
  NLPHL: cervical, axillary, and inguinal nodes.
  CHL: cervical, mediastinal, and axillary nodes.
  Extranodal dissemination is rare in both subtypes.
- **Treatment**
  Chemotherapy, radiation
- **Outcome**
  NLPHL: patients with low stage disease have a 10 year survival round 80%.
  CHL: Over 80% of patients are cured.

Microscopy

NLPHL: The smears show a large number of predominantly mature lymphoid cells and histiocytes some of which are of epithelioid type. The tumor cells are large with a scant, poorly visible cytoplasm and an irregular nucleus with small nucleoli (Fig. 1).

CHL: Most cases show an abundant non-neoplastic lymphoid population of mostly small mature lymphoid cells together with eosinophils, neutrophils, plasma cells, and histiocytes. Fibroblasts and collagen fragments are often seen in the nodular sclerosis type. There are two types of large tumor cells: the mononuclear Hodgkin cell with a large nucleus and a large nucleolus. The basophilic cytoplasm is rich. The second cell type is the binucleated Reed-Sternberg cell. These cells have nuclei with large nucleoli and a rich cytoplasm (Fig. 2).

Immunophenotype

NLPHL: CD45, CD20, CD79a, OCT-2, and BOB1 positive. CD15 and CD30 negative.

CHL: CD30 and often MUM1 and CD15 positive. CD45, OCT-2, and BOB1 negative.

Molecular Features

NLPHL: clonal rearrangement of the immunoglobulin gene

CHL: clonal rearrangement of the immunoglobulin gene

Differential Diagnosis

NLPHL: reactive lymphadenitis, T-cell rich B-cell lymphoma, early phase of metastatic carcinoma or melanoma.

CHL: variants of reactive lymphadenitis, T-cell rich B-cell lymphoma, anaplastic large cell lymphoma, metastasis of carcinoma or melanoma.

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Human Papillomavirus

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Synonyms

Human Papilloma Virus; HPV

Definition

HPV is a double stranded DNA virus of approximately 55 nm and have an icosahedral protein capsid comprising of 72 capsomeres. It is a member of Papillomaviridae family and affects the cells of cutaneous and mucosal epithelia (Centers for Disease Control and Prevention (CDC) 2008). The genome of HPV is circular and contains 7,500–8,000 base pairs. More than 150 different types of HPV have been known till date, of which over 40 infect the anogenital region. Some HPV types have been recognized as high-risk or oncogenic HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and some as low-risk HPV (6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81) (Koushik and Franco 2006). HPV 16 and 18 are the most frequently associated subtypes with cervical cancer and almost all cervical cancers are caused by HPV (Waloomers et al. 1999). Majority of HPV infections especially in young women are temporary and the viral infection is cleared within a period of 2 years. Persistent infection with "high-risk HPV" in a minority of women may progress to pre-cancerous lesions of the cervix (cervical intra-epithelial neoplasia CIN) and can lead to cervical cancer (Schiffman and Castle 2003). These pre-cancerous lesions of the cervix can be detected by cervical cytology smears, which is the basis of cervical screening programs in developed countries. The cervical smear can be taken by conventional method or by liquid-based cytology (LBC). Screening by Pap smears has reduced mortality due to cervical cancer in the developed world. Detection of high-risk HPV by in situ hybridization or polymerase chain reaction can also help in screening or triaging women, who need close follow-up or treatment of the pre-cancerous lesions of the cervix.

Clinical Features

- **Incidence**
  The cervical cancer is the twelfth most common cancer in the world (Armstrong 2010) and affects approximately 16 per 10,000 women

per year (Globocan 2002 database 2008). Due to the organized cervical screening programs in the developing world, majority of cervical cancers occur in the developing countries. According to Cancer Research UK Cervical cancer statistics, the UK's European age-standardized mortality is 2.4/100,000 per year (2007) (http://info.cancerresearchuk.org/cancerstats/types/cervix/incidence/). HPV affects approximately 80% of all sexually active people, and women who have multiple sexual partners are at greater risk (Marrazzo et al. 2001).

- **Age**
  Majority of sexually active males and females acquire genital HPV infection at sometime in their lives (Baseman and Koutsky 2005), and therefore, the HPV infection may be seen at any age, with majority of infections in 14–65 years of age. Highest prevalence is seen in females of less than 25 years of age (Franceschi et al. 2006).
- **Risk Factors**
  Various risk factors for HPV infection include number of sexual partners, age at first intercourse, parity, and oral contraceptive use (Koushik and Franco 2006).
- **Sex and Site**
  HPV infection can occur in both males and females and can affect skin and mucosal epithelium. Low-risk HPV subtypes may cause cutaneous warts or papillomas and high-risk types are known to cause cervical cancer, anal cancer, vulvar cancer, vaginal cancer, oropharyngeal and esophageal cancers, and penile cancer (Parkin 2006).
- **Treatment and Outcome**
  There is no specific treatment to clear the HPV infection at present. Immune system of human body can clear infection within 2 years in almost 90% cases (Centers for Disease Control and Prevention (CDC) 2008). Persistent high-risk HPV infection leads to pre-cancerous lesions which can progress to cancer.

HPV Infection: Natural History

HPV infects the basal cells of stratified epithelium, when it gets exposed as a result of epithelial trauma or micro-abrasions. The HPV genome comprises of six early proteins (E1, E2, E3, E4, E6, and E7) and two late (L1 and L2) proteins (Ganguly and Parihar 2009). Once the host cell is infected, E1 and E2 are expressed. After integration of HPV DNA into the host genome, the function of E2 gets disrupted, which prevents repression of E6/E7. The expression of E6 and E7 is associated with cervical cancer, and it produces the proteins that interfere with tumor-suppressor genes controlling the cell cycle. Viral DNA gets integrated into the host's genome, and E6 and E7 gets upregulated. E7 forms a complex with cell growth regulator retinoblastoma (rb) protein and causes uncontrolled cell proliferation (Munger et al. 2001). E6 binds to p53 protein, promoting its degradation, which in turn leads to loss of DNA repair function and the apoptosis of the cell is prevented (Mantovani and Banks 2001). The infected cell becomes susceptible to mutations and genomic instability.

Laboratory Detection

HPV detection techniques include immunocytochemical techniques, dot blot, southern blot, in situ hybridization, polymerase chain reaction (PCR) techniques, and genotyping methods (Gupta et al. 2010; Farthing et al. 1994; Ergünay 2008). HPV genotyping can be done by various commercially available assays (Castle et al. 2007). Polymerase chain reaction has also been used for viral load determination (Hubbard 2003).

HPV Vaccine FDA (Food and Drug Administration) approved HPV vaccines are available for prevention against HPV infection (http://en.wikipedia.org/wiki/Human_papillomavirus). These vaccines protect against the initial infection by HPV 16 and HPV 18.

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Human Polyomavirus, Cytological Findings

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Synonyms

BK virus; Decoy cells

Definition

Polyomavirus infects most people subclinically during childhood, most probably via an upper respiratory or gastrointestinal infection. In most immunocompetent individuals, the infection remains asymptomatic, despite clinically silent periods of self-limiting viral reactivation during slight changes in immune surveillance. Since the urothelium is a common site of viral latency, reactivation often occurs in the urothelial cells layer with shedding viral inclusion-bearing cells (so-called decoy cells) into the urine. The first strain of human polyomavirus was isolated from the urine in 1971 and named "BK-polyomavirus" strain after the initials of the patient. Decoy cells contain mainly BK virus, less commonly JC virus. In immunocompromised patients, BK reactivation can lead to severe disease, including polyoma virus-associated nephropathy (BKN) in kidney transplant patients and hemorrhagic cystitis in patients with allogenic hematopoietic stem cell transplantation. In addition, there might also be an association between BKV infection and urothelial carcinomas, since BKV-positive urothelial carcinomas exist. Urine cytology serves

as a simple, cheap, and highly sensitive method for the early identification patients at risk for BKN after kidney transplantation, together with BKV DNA analysis by quantitative PCR in the serum. BKV causes a spectrum of morphologic changes of tubular and urothelial cells. The transient appearance of rare decoy cells can safely be regarded clinically irrelevant. A semiquantitative count of >5 decoy cells/10 HPF (magnification × 400) in smears of urinary sediments is considered significant. In cytospin specimens, the cutoff can be raised to 10 decoy cells/10 HPF, as cytospins typically show a higher cell density than the smears from urinary sediments. In such high-risk patients, further analyses are performed including PCR analysis for detection of virus DNA in the peripheral blood and/or renal biopsy. The negative predictive value of cytology for decoy cells in voided urine is around 99% ("no decoy cells, no BKN"). In contrast, the positive predictive value can be as low as 27%. The positive predictive value can be further increased to over 90% by taking additional parameters into consideration: (a) a "dirty" cytological background, (b) decoy cell shedding in the setting of allograft dysfunction, (c) extended and persistent decoy cell shedding over more than 6 weeks, and (d) the detection of decoy cell casts. Screening for decoy cells in voided urines plays a central role in an algorithmic approach. Accordingly, all renal transplant recipients should be screened for BKV replication in the urine (1) every 3 months during the first 2 years posttransplant, (2) when allograft dysfunction is noted, and (3) when allograft biopsy is performed. A positive screening result should be confirmed in <4 weeks and assessed by quantitative assays (e.g., BKV DNA or RNA load in plasma or urine). Definitive diagnosis of BK virus-associated nephropathy (BKN) requires allograft biopsy.

Clinical Features

- **Incidence**
  Based on the detection of decoy cells, transient and asymptomatic reactivation of BKV is seen in no more than 0.5% of all urine cytology specimens. A high prevalence of decoy cell shedding is found in pregnant women (3%), patients suffering from cancer (13%), and diabetes mellitus (3%), as well as in renal allograft (23%) and pancreas transplant (11%) recipients. The prevalence of biopsy-proven BKN in patients with renal transplantation is approximately 3%, while BKV-associated hemorrhagic cystitis occurs in 10–15% of patients with allogenic hematopoietic stem cell transplantation.
- **Age**
  Mostly in adults, but no age predilection.
- **Sex**
  No gender predilection.
- **Site**
  PV infection or reactivation primarily occurs in urothelium of the upper urinary tract. It is believed that infection of the renal tubular cells is mainly by systemic spread through the blood rather than by direct transgression from the renal pelvis. However, an ascending route of infection with spreading of PV replication from urothelial cells to collecting ducts and tubular epithelial cells is equally possible.
- **Treatment**
  There is no effective antiviral therapy. Treatment relies on reconstitution of the immune function by reducing or changing the immunosuppressive medication.
- **Outcome**
  If the BKV reactivation is detected early, loss of the kidney transplant because of BKN can be prevented in most patients.

Macroscopy

The second morning midstream voided urine specimen is best suited as the high level of cellular degeneration limits the first morning urine. Fresh urine specimens should be promptly transported to the cytology laboratory for immediate processing. Alternatively, and more frequently used, the voided urine can be diluted with an equal volume of 50% ethyl alcohol, optionally with added 2% Carbowax, to preserve the cells for up

to 3 days and prevent bacterial overgrowth during transport. Conventional cytospins on coated glass slides are the preferred preparation method. The slides should be immediately fixed in alcohol and stained according to Papanicolaou to highlight the typical nuclear features of decoy cells.

Microscopy

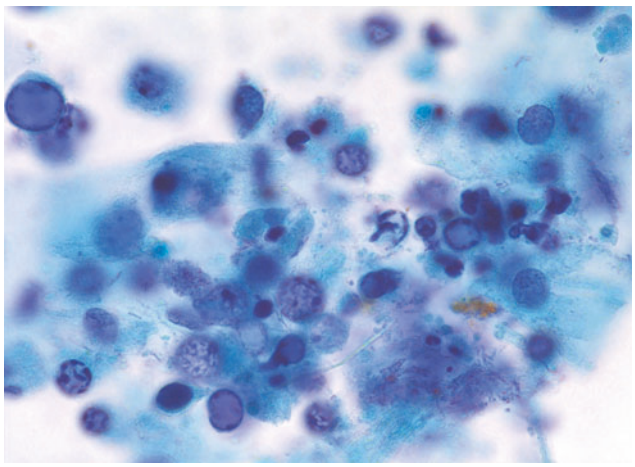
BK virus infection of tubular and urothelial cells covers a wide spectrum of morphologic changes. Using the Papanicolaou stain in cytology specimens, four major morphologic types of "decoy cells" can be distinguished based on nuclear characteristics: (a) *classic decoy cell*: round, enlarged nuclei with large, amorphous nuclear ground-glass inclusion and a dense peripheral rim of compressed chromatin. (b) *Granular type*: cells with granular inclusion bodies in the nucleus surrounded by a halo. It can be mistaken for cytomegalovirus infection. This subtype is not very common. (c) *Polymorph-hyperchromatic type* (carcinoma-like): dark and polymorphic nuclei with coarsely granulated chromatin, reminding of urothelial cancer cell. These cells may be bi- or trinucleate. (d) *Vesicular type*: large, round, and vesicular nucleus with a clear background. A peculiar network of clumped chromatin with a reticular pattern spans the three-dimensional nucleus like a spider web. Nucleoli may be prominent. Typical examples of decoy cells are shown in Fig. 1.

The origin of decoy cells cannot be easily determined based on morphological grounds. It seems likely that they would commonly originate from the urothelium, particularly in healthy and asymptomatic patients. In contrast, one would assume that decoy cells from immunocompromised patients with BKN, decoy cells likely originate also from the renal parenchyma.

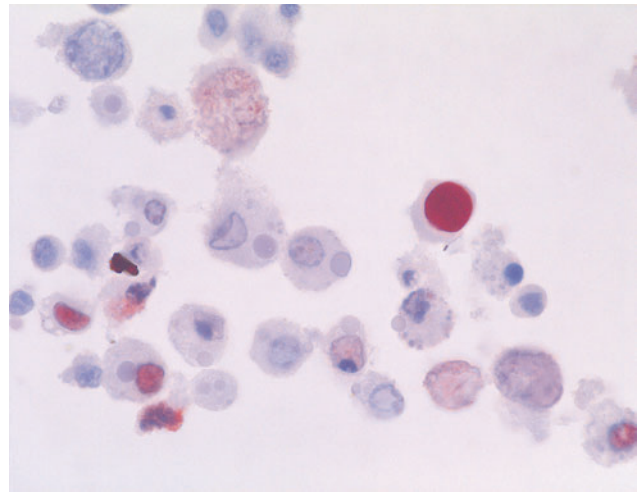
Cytospins from voided urines showing the whole range of decoy cell morphology can be viewed online through the virtual microscope at http://vmic.unibas.ch/patho/seminar/index.html.

Immunophenotype

In most cases, diagnosis of decoy cells is straightforward for those who are familiar with the characteristic morphological features. Immunocytochemistry can be used to confirm the diagnosis in equivocal cases (SV40 T Ag, Calbiochem) as illustrated in Fig. 2. The intranuclear virus particles can also be visualized by electron microscopy. The utility of the highly sensitive PCR analysis of voided urine is controversial as it often detects irrelevant levels of BKV activation. Interestingly, BKV infection of the urothelial cells leads to nuclear overexpression of p53.



Human Polyomavirus, Cytological Findings, Fig. 1 Morphological spectrum of decoy cells in voided urines (Papanicolaou, ×600)



Human Polyomavirus, Cytological Findings, Fig. 2 SV40-positive decoy cells (immunocytochemistry, ×600)

Molecular Features

Decoy cells are almost invariably aneuploidy by DNA cytometry due to the random intranuclear accumulation of viral DNA. However, fluorescence in situ hybridization using probes specific for human DNA shows normal copy numbers, identifying the decoy cells as benign.

Differential Diagnosis

As the term decoy implies, these polyomavirus-infected cells can masquerade as carcinoma cells due to their large nuclei, the dark chromatin, and high nuclear-cytoplasmic ratio. There are several clues to the correct diagnosis. Importantly, the nuclei mostly appear like balloons with a smooth regular contour, and there are almost always at least a few classical decoy cells with the typical ground-glass nuclear quality and an irregular peripheral density representing the compressed human chromatin. Typically, these classical decoy cells reveal a taillike cytoplasm that gives the cell a comet-like appearance. Above all, awareness of decoy cells is the major key to avoiding a misdiagnosis of urothelial carcinoma. Notably, the presence of decoy cells does not exclude urothelial carcinoma, since rare BKV-positive urothelial carcinomas exist.

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Hürthle Cell Tumors, Cytological Findings

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Synonyms

Follicular neoplasm/Hürthle cell type; Follicular neoplasm/oncocytic type; Oncocytic neoplasm; Oncocytic tumor

Definition

Follicular neoplasm/Hürthle cell type or Hürthle cell tumor concerns proliferation of exclusive or quite exclusive oncocytes; it includes oncocytic adenoma as well as oncocytic carcinoma. The Hürthle cell tumors belong to the category of follicular tumors and should not include the papillary carcinoma/Hürthle cell or oncocytic variant.

Clinical Features

- **Incidence**
  The estimated incidences for Hürthle cell tumors are 2% for women and less than 1% for men.
- **Age**
  The prevalence of the Hürthle cell tumors increases with age, and is mostly observed in people over 50.
- **Sex**
  The sex ratio is F:M: 6.5:3.5. The risk of cancer is twice as high for men than for women and increases with older people.
- **Site**
  The lesion may be found anywhere in the thyroid gland.
- **Treatment**
  The treatment depends on the final histological diagnosis. Oncocytic tumors are usually removed surgically since there are no cytological criteria on Fine Needle Aspiration (FNA) to distinguish one from the other. For the oncocytic carcinoma, total thyroïdectomy is required, often with neck nodes dissection, followed or not by radioiodine treatment, depending on the stage of the tumor.
- **Outcome**
  The prognosis is excellent for oncocytic adenoma since it is a benign tumor. It is variable for oncocytic carcinoma, depending on the patient's age, the size and the histological stage of the tumor as well as the existence or not of lymph nodes metastases; older people, size higher than 4 cm, pT3 and metastases represent adverse factors. Till recently, oncocytic carcinoma has been considered as more aggressive than follicular carcinoma; but more recent series have shown equivalent survival rates for the same stage of disease.

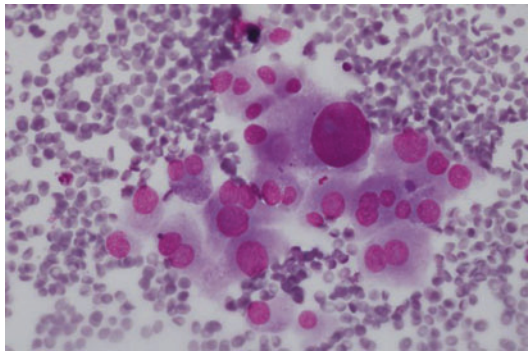
Macroscopy

Hürthle cell adenoma is mostly a unique tumor, well delimited by a very thin and quite unapparent capsule and with a central fibrous scar. Its color is typically a mahogany-brown but may be ochre. Some hemorrhagic foci and cysts may be observed. Hürthle cell carcinoma does not really differ from the adenoma except in case of obvious invasion.

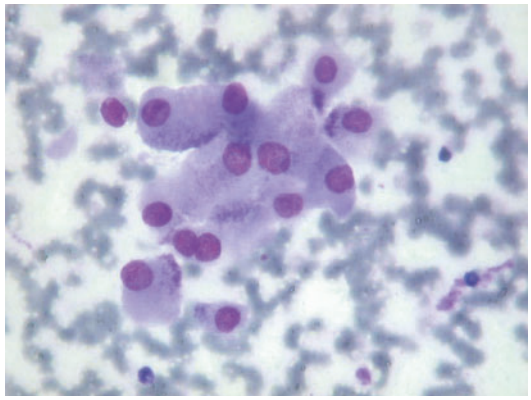
Cytological Findings

Hürthle cell or oncocytic tumors do not differ on FNA whatever benign or malignant. All or quite all the epithelial cells observed on the slides are oncocytes. Typically, oncocytes are large cells with an abundant and granular cytoplasm including sometimes some blue-purple cytoplasmic granulations, with MGG staining, which represent the numerous mitochondria characteristic for these cells. On Papanicolaou staining, the cytoplasm appears sometimes amphophilic. The nuclei are usually enlarged, eccentric, with large or prominent nucleoli. A clear anisokaryosis is frequent, as well as bi- or multinucleated oncocytes. Colloid and histiocytes may be observed too. Whatever the cells atypia encountered none is significant for malignancy (Figs. 1 and 2).

On liquid-based cytology, oncocytes are often quite different due to the shrinkage of the cells with the alcoholic fixatives present in the liquid medium. Nuclei are reduced in size, more oval than round, and the cytoplasm is less typically granular (Fig. 3).



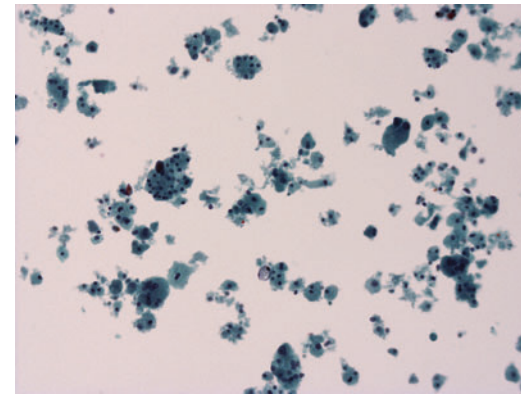
Hürthle Cell Tumors, Cytological Findings, Fig. 1 A loosely group of large cells with a basophilic, less or more granular cytoplasm; notice the numerous binucleated cells as well as the marked anisokaryosis (conventional slide; MGG staining × 40)



Hürthle Cell Tumors, Cytological Findings, Fig. 2 A group of typical oncocytes with a large basophilic and granular cytoplasm including some *dark blue* granulations. The nuclei are eccentric and round (conventional slide; MGG staining × 40)

Immunophenotype

The neoplastic cells of the benign and malignant Hürthle cell tumors are both positive with TTF1, CK7, and thyroglobulin antibodies but negative for CK20. Other antibodies useful for follicular tumors are not recommended in Hürthle cell tumors due to the wide variety of results in terms of positivity or negativity. To summarize, immunocytochemistry/histochemistry is not really useful in oncocytic tumors except to recognize the thyroid origin.



Hürthle Cell Tumors, Cytological Findings, Fig. 3 Some dispersed large cells with a more or less granular cytoplasm and eccentric nuclei, often oval and of small size; some cells include two or three nuclei. These cells are typical oncocytes on LBC (LBC; Hologic®; Papanicolaou staining × 25)

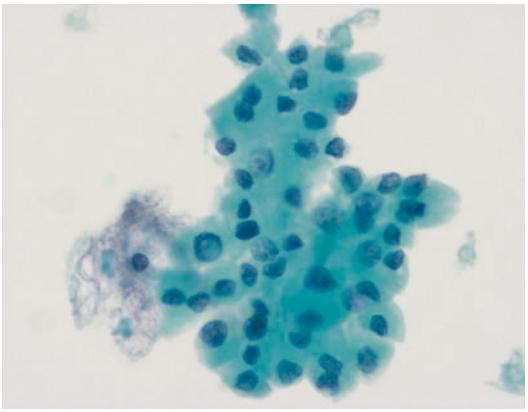
Nevertheless, some authors have reported the diagnostic performance of two antibodies, cyclin D1 and cyclin D3 whose over-expression could predict malignant behavior of oncocytic tumors.

Molecular Features

PAX8-PPARγ fusion is rarely found in Hürthle cell carcinoma, and they harbor neither RET/PTC rearrangements nor BRAF mutations; MiR-885-5p is highly upregulated in oncocytic follicular carcinomas and other miRNA are being tested in oncocytic tumors and could serve as a useful diagnostic markers.

Differential Diagnosis

Oncocytic tumors are often easy to recognize cytologically but may be challenging in some cases. The main differential diagnosis concerns the papillary carcinoma/oncocytic variant; nevertheless, nuclear atypia such as nuclear grooves, elongated nuclei, and/or pseudoinclusions are usually present, leading to the right diagnosis (Fig. 4). Recently, a new entity, the so-called EPONs tumors (encapsulated papillary oncocytic neoplasm), has been described.



Hürthle Cell Tumors, Cytological Findings, Fig. 4 A sheet of large cells sometimes binucleated, with enlarged, mostly round nuclei; but there is one pseudoinclusion and some nuclei with irregular borders. These features suggest a papillary carcinoma/oncocytic variant (LBC; Hologic®; Papanicolaou staining × 40)

It is the association of papillary arrangement with oncocytic cells. These tumors seem to be CD56 positive, but BRAF V600 and RET/PTC rearrangements are negative.

It is necessary in some cases to discuss medullary carcinoma, especially when the oncocytic cells are less large. The oncocytes and the cells of the medullary carcinoma share granular cytoplasm and bi-/multinucleated cells. Notice that in medullary carcinoma, with MGG staining, the cytoplasm may include eosinophilic granulations and some eosinopilic drops of amyloid in the background.

Finally, histiocytes may also look like oncocytes when they are not vacuolated and do not include some hemosiderin pigment. CD68 immunocytochemistry may be helpful.

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Hyalinizing Trabecular Adenoma, Cytological Findings

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Synonyms

Papillary carcinoma/hyalinizing variant; Paraganglioma-like adenoma

Definition

Hyalinizing trabecular adenoma (HTA) of the thyroid gland has been firstly described in 1905 and is well known by pathologists. As explained by its descriptive name, this tumor is composed of follicular cells in a trabecular arrangement and by a hyalinized fibrosis.

Clinical Features

- **Incidence**
  This tumor is quite rare.

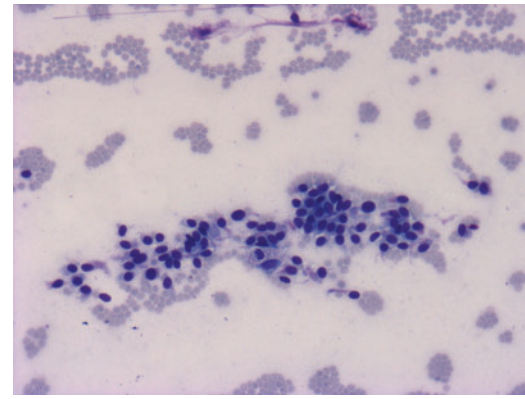
- **Age**
  It may appear at any age of the life, but usually not before 30 years old and more often in the second half of life.
- **Sex**
  Like for the other thyroid tumors, it appears more frequently in females than in men.
- **Site**
  The lesion may be found anywhere in the thyroid gland.
- **Treatment**
  Usually, when discovered, this tumor will be removed due to its challenging cytological diagnosis (see below).
- **Outcome**
  There is mostly no adverse prognosis since HTA is considered as a benign tumor. Nevertheless, some rare malignant cases have been described in the literature.

Macroscopy

Macroscopically HTA is not different from follicular adenoma. It is a well-delimited tumor; color is yellow to orange or pink; the size is variable from less than 1 cm to more than 7 cms. Some cysts may be seen.

Cytological Findings

Typically, HTA is composed of cells with variable sizes and shapes. The follicular cells may be round or polygonal or spindle-shaped. The cytoplasm is more or less abundant, sometimes amphophilic like the oncocytes. The nuclei may be round or oval but often show the same atypia as the nuclei in papillary carcinoma, i.e., pseudoinclusions, grooves, and some overlapping. Psammomas may be present; in the most typical cases, there are some amounts of hyaline material, sometimes surrounded by the cells, pink on MGG staining (Fig. 2). There is no colloid, and the cellularity is usually poor or moderate due to the fibrosis. Considering these cytological characteristics, it is easy to understand that HTA is a challenging diagnosis on FNA.



Hyalinizing Trabecular Adenoma, Cytological Findings, Fig. 1 Some spindle-shaped cells with oval nuclei that might suggest medullary carcinoma but belong to an HTA (conventional slide; MGG staining × 25)

Immunophenotype

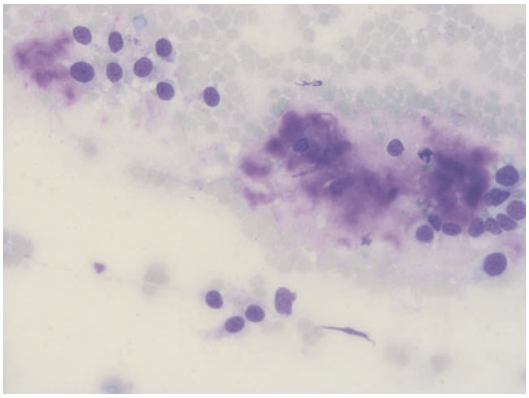
The neoplastic cells are positive for TTF1, thyroglobulin, and MIB-1, and they may be positive for p S 100, chromogranin, NSE, CK19, and Galectin 3. They are usually negative for HBME1, calcitonin, and CEA. The MIB-1 positivity is a cytoplasmic positivity.

Molecular Features

The same RET/PTC translocations which are observed in about 20–40% of papillary carcinoma have been found in HTA supplying the debates concerning the benign or malignant behavior of this tumor; therefore, it is sometimes considered as an encapsulated papillary carcinoma. Nevertheless, further studies have shown that HTA is BRAF V600 negative, underlining its difference with about 50% of the papillary carcinoma.

Differential Diagnosis

HTA should be differentiated from papillary carcinoma essentially. In this way, immunocytochemistry is essential with the MIB-1 cytoplasmic positivity found in the HTA. But morphologically the diagnosis may be quite difficult and often only



Hyalinizing Trabecular Adenoma, Cytological Findings, Fig. 2 Some polygonal cells with discrete nuclear atypia and dense amount of metachromatic, pink collagen in the background (conventional slide; MGG staining 40)

suggested but not asserted, obviously leading to a surgical control. In some cases, cells may also mimic either cells of medullary carcinoma or cells of Hürthle cells tumors due to the presence of cells with basophilic cytoplasm on MGG staining, of spindle-shaped cells, and pink amounts of material in the background (Fig. 1). Immunocytochemistry is essential again and offers the only opportunity to solve the difficulties.

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Infectious Mononucleosis, Cytological Findings

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Synonyms

Epstein-Barr virus infection; Glandular fever, "kissing disease"

Definition

The clinical syndrome of infectious mononucleosis is an acute, primary infection by Epstein-Barr virus (EBV) which may lead to lymphadenopathy and splenomegaly. Usually, the disease is characterized by pharyngitis, fever, cervical or generalized lymphadenopathy, and atypical lymphocytosis.

Clinical Features

- **Incidence**
  About 1 in 200 people in USA develops mononucleosis each year.
- **Age**
  Often affects children and young adolescents.
- **Sex**
  There is no sex predilection.
- **Site**
  Cervical or generalized lymphadenopathy.
- **Treatment**
  There is no specific treatment or therapy.
- **Outcome**
  Acute onset and a benign, self-limited course.

Microscopy: Cytological Findings

Cytologically, the lymph node aspirates show a great number of large immunoblastic lymphocytes than seen in the reactive lymph nodes of nonspecific type. The polymorphic immunoblastic proliferations in lymph node cytology are suggestive of infectious mononucleosis (Fig. 1). In some cases, cells with double nuclei which mimic Reed-Sternberg cells are present (Fig. 2).

Immunophenotype

The large immunoblastic lymphocytes are both B- and T-cells and they express CD30. B-cells are polytypic (both kappa and lambda).

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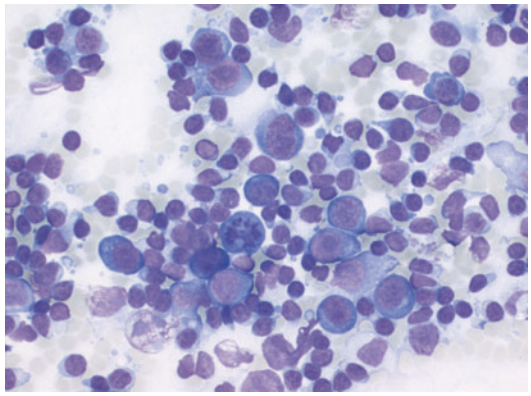
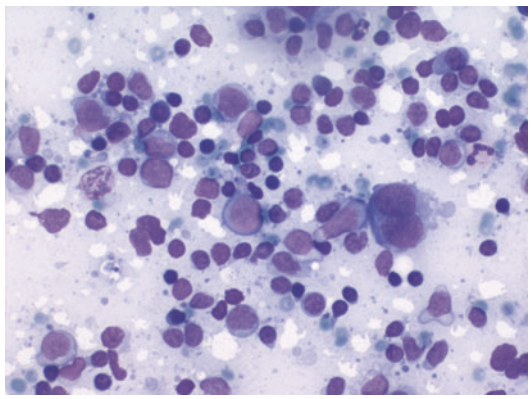


Infectious Mononucleosis, Cytological Findings, Fig. 1 Mononucleosis: Aspirate from lymph node shows small mature lymphocytes and large immunoblasts with prominent nucleoli and basophilic cytoplasm. Centroblasts with round nuclei and sparse *gray-blue* cytoplasm are also present. Mitosis is found. MGG



Infectious Mononucleosis, Cytological Findings, Fig. 2 Mononucleosis: a mixed population of small-medium sized cells with sparse cytoplasm, immunoblasts, and centroblasts. One large cell with double nuclei mimicking a Reed-Sternberg cell. MGG

Differential Diagnosis

Viral lymphadenitis, postvaccinal lymphadenitis, and malignant lymphoma.

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Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

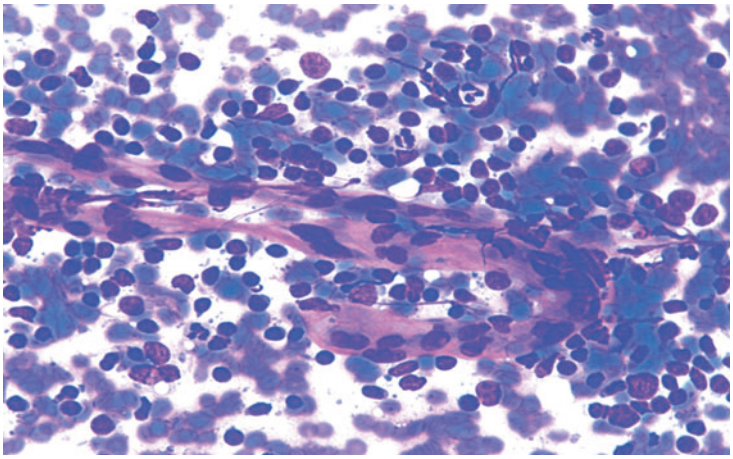
► Inflammatory pseudo tumor represents a spectrum of orbital space-occupying inflammatory disorders.

Clinical Features

- **Incidence**
  Rare, accounts for 5–18% of orbital masses.
- **Age**
  Middle-aged patients.
- **Sex**
  No gender predilection.
- **Site**
  Orbit.
- **Treatment**
  Surgery.
- **Outcome**
  Favorable.

Macroscopy

Orbital mass.



Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings, Fig. 1 Orbital FNC of an inflammatory pseudotumor: inflammatory cells and fibrous cells are interspersed in the smear (Diff-Quik stain, 270×)

Microscopy

Acute inflammatory pseudotumors have a quite specific clinical and instrumental presentation, whereas chronic idiopathic pseudotumors are more subtle. They may be formed by fibrosis, capillary vessels, inflammatory cells, and granulomatous patterns (Fig. 1) and may be cytologically indistinguishable from other specific or nonspecific granulomatous processes. More complex pseudotumors may show, in addition to ambiguous imaging, a similar cytological pattern to those of lymph nodes and the same problems of differential diagnosis with lymphomas. Corresponding smears are composed of lymphoid cells in various stages of maturation, such as in the smears of hyperplastic lymph nodes. ICC and FC may be successfully used to distinguish lymphoid pseudotumor from non-Hodgkin lymphoma provided that the yield of cells is sufficient.

Differential Diagnosis

Lymphoproliferative disorder.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Lacrimal Gland Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
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- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
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- ▶ Orbital Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings

- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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K

Kikuchi Lymphadenitis, Cytological Findings

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Synonyms

Histiocytic necrotizing lymphadenitis; Kikuchi-Fujimoto disease; Subacute necrotizing lymphadenitis

Definition

Kikuchi-Fujimoto disease is an uncommon, benign, self-limiting disease of unknown etiology which was first described independently in 1972 in Japan by Kikuchi and Fujimoto et al. It is characterized by a solitary, tender, cervical subacute lymphadenopathy in young female adults with low-grade fever.

Clinical Features

- **Incidence**
  The disease has worldwide distribution and is more frequently encountered in individuals of Asian or Far Eastern origin.
- **Age**
  Wide range of age, but often young adult with means age of 30 years.
- **Sex**
  Slight predominance of female.
- **Site**
  Typically involves the cervical lymph nodes, often posterior neck nodes but axillary nodes have been described.
- **Treatment**
  Because of its unknown etiology, a specific treatment is currently unavailable. Owing to the association with SLE, patients with Kikuchi lymphadenitis must undergo a systemic survey and long-term follow-up to evaluate subsequent development of SLE.
- **Outcome**
  Benign and self-limited disease.

Microscopy: Cytological Findings

A polymorphous lymphoid population with abundant apoptotic, karyorrhectic debris and phagocytic histiocytes. Many of the macrophages present an eccentrically placed, crescent formed nucleus and ingested intracytoplasmic apoptotic nuclear debris (Figs. 1 and 2). The lymphoid cell population consists of small mature lymphocytes and same larger transformed cells of immunoblastic type. Neutrophils are notably absent.

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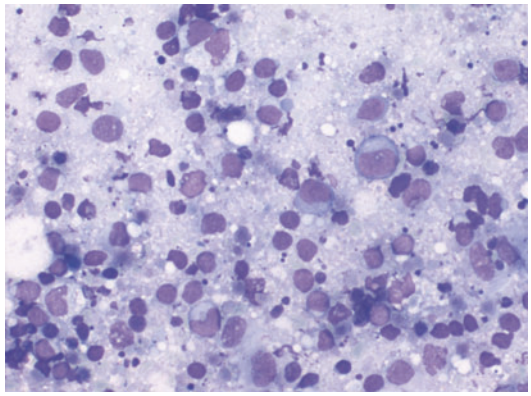
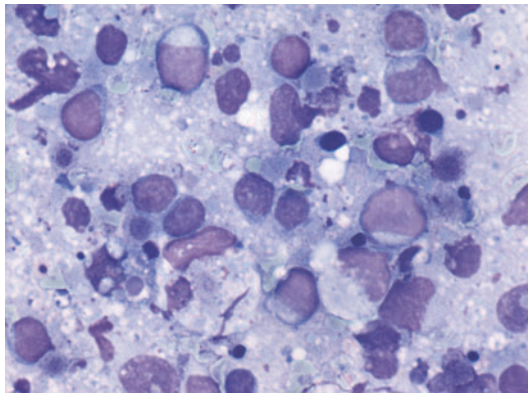


Kikuchi Lymphadenitis, Cytological Findings, Fig. 1 Kikuchi lymphadenitis: cell debris and mixed lymphatic cell population with the presence of few immature large cells. Some phagocytic histiocytes and monocytic cells. MGG



Kikuchi Lymphadenitis, Cytological Findings, Fig. 2 Kikuchi lymphadenitis: mixed lymphoid cell population, cell debris, and macrophages. MGG

Immunophenotype

There is an abundance of T-cells with predominance of CD8-positive over CD4-positive T-cells.

Molecular Features

No chromosomal changes known.

Differential Diagnosis

Lymphoma, toxoplasmic lymphadenitis, tuberculous lymphadenitis, cat scratch disease, and lymphadenitis associated with systemic lupus erythematosus.

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L

Lacrimal Gland Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Description

Almost all the salivary tumors have been observed in the lachrymal glands with the same cytological features.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
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- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
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Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

The most frequently reported malignant tumors are ▶ adenoid cystic carcinoma, ▶ mucoepidermoid carcinoma, and malignant tumor ex pleomorphic adenoma (malignant mixed tumor).

Clinical Features

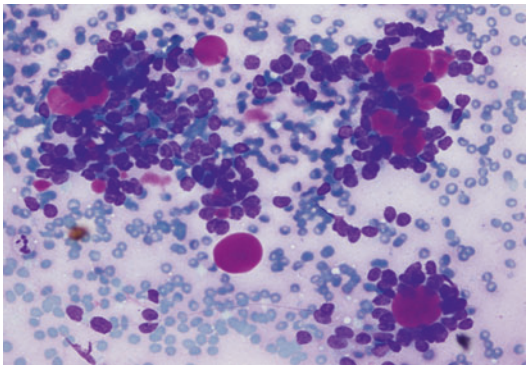
- **Incidence**
  Rare.
- **Age**
  Young middle age.
- **Sex**
  No gender predilection.
- **Site**
  Upper outer orbital cantus.
- **Treatment**
  Surgery and radiotherapy.
- **Outcome**
  Poor overall prognosis.

Macroscopy

Oval solid tumor and possible calcifications.

Microscopy

FNC of these entities have been reported, and the corresponding cytological features are those described in salivary gland tumors. ▶ Adenoid cystic carcinoma is characterized by small round cells with dense chromatin, without nucleoli. These cells lack cytoplasm and are often organized in tubular structures. MGG or Diff-Quik may help to recognize the metachromatic red-magenta "cylinders" (Fig. 1). ▶ Mucoepidermoid carcinoma features range from extremely well-differentiated forms in which a smear shows only abundant mucus and few well-differentiated mucoid cells to the poorly differentiated squamous type. Therefore, differential diagnosis may be pointed out with mucocele or mucoid cyst and pure squamous cell carcinoma, respectively. The malignant tumor ex pleomorphic adenoma generally lacks specific cytological features, and the possibility of a diagnosis from FNC samples depends on the detection of unequivocal images of concomitant ▶ pleomorphic adenoma in the smears; a history of a long-standing lachrymal



Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings, Fig. 1 FNC of an adenoid cystic carcinoma of lacrimal gland. Tumoral, small monomorphic cells with dense nuclei without cytoplasms encircling "cylinders" of reddish metachromatic matrix (Diff-Quik stain 270×)

mass may further support the cytological diagnosis.

Differential Diagnosis

▶ Adenoid cystic carcinoma, ▶ mucoepidermoid carcinoma, and malignant tumor ex pleomorphic adenoma (malignant mixed tumor).

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Lacrimal Gland Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Langerhans Cell Histiocytosis, Cytological Findings

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Synonyms

Multiorgan involvement: Lettere-Siwe disease; Multiple lesions: Hans-Schüller Christian disease; Solitary lesions: eosinophilic granuloma, histiocytosis X

Definition

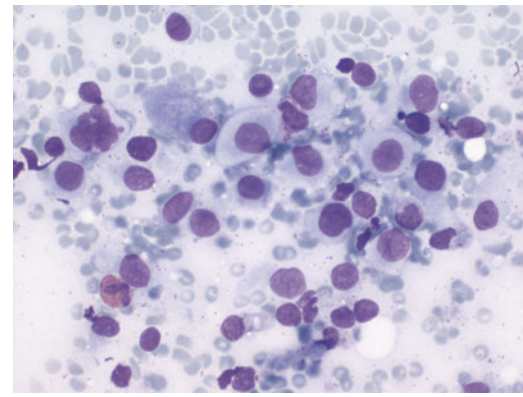
Neoplastic proliferation of Langerhans cells.

Clinical Features

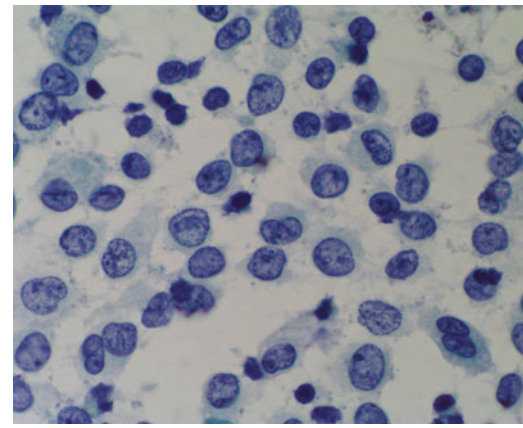
- **Incidence**
  5/1,000,000.
- **Age**
  All ages are affected but most patients are under 30 years.
- **Sex**
  Males twice as common as females.
- **Site**
  Any bone may be involved in the solitary form. The multiorgan disease may involve skin, lungs, liver, and bone marrow.
- **Treatment**
  Chemotherapy, radiation, and surgery.
- **Outcome**
  The unifocal disease has an excellent prognosis while the multisystemic patients have a survival around 50%.

Microscopy

The tumor cells of Langerhans cell histiocytosis have a rich gray-blue cytoplasm with a grooved/indented nucleus with fine chromatin. Granulocytes, lymphocytes, and eosinophils are always found (Figs. 1 and 2).



Langerhans Cell Histiocytosis, Cytological Findings, Fig. 1 Langerhans cell histiocytosis: histiocytes with round to oval nuclei often with a nuclear fold and distinct rich cytoplasm. Neutrophils and eosinophils are always present. MGG



Langerhans Cell Histiocytosis, Cytological Findings, Fig. 2 Langerhans cell histiocytosis: histiocytes with round to oval nuclei often with a nuclear fold. Papanicolaou

Immunophenotype

Vimentin, CD1a, and S-100 positivity.

Molecular Features

No consistent changes are found.

Differential Diagnosis

Nonspecific inflammatory disease.

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Large Cell Neuroendocrine Carcinoma, Cytological Findings

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Definition

Large cell neuroendocrine carcinoma (LCNEC) of the lung is an aggressive, non-small cell, high-grade neuroendocrine carcinoma occurring in cigarette smokers. LCNEC is defined by histologic criteria, which were established on surgical specimens. The diagnosis requires (1) neuroendocrine morphology (organoid nests, peripheral palisading, rosettes, or trabeculae), (2) expression of at least one neuroendocrine marker (CD56, chromogranin, or synaptophysin), (3) non-small cell cytological features, (4) a high mitotic rate (>10 mitoses per 2 mm^2), and (5) necrosis.

On small biopsies and cytology all five diagnostic criteria, especially the neuroendocrine morphology, are rarely identifiable. Therefore it is suggested to phrase the diagnosis as "non-small cell lung carcinoma (NSCLC) with features suggestive of neuroendocrine morphology and with positive neuroendocrine markers, possible LCNEC."

Clinical Features

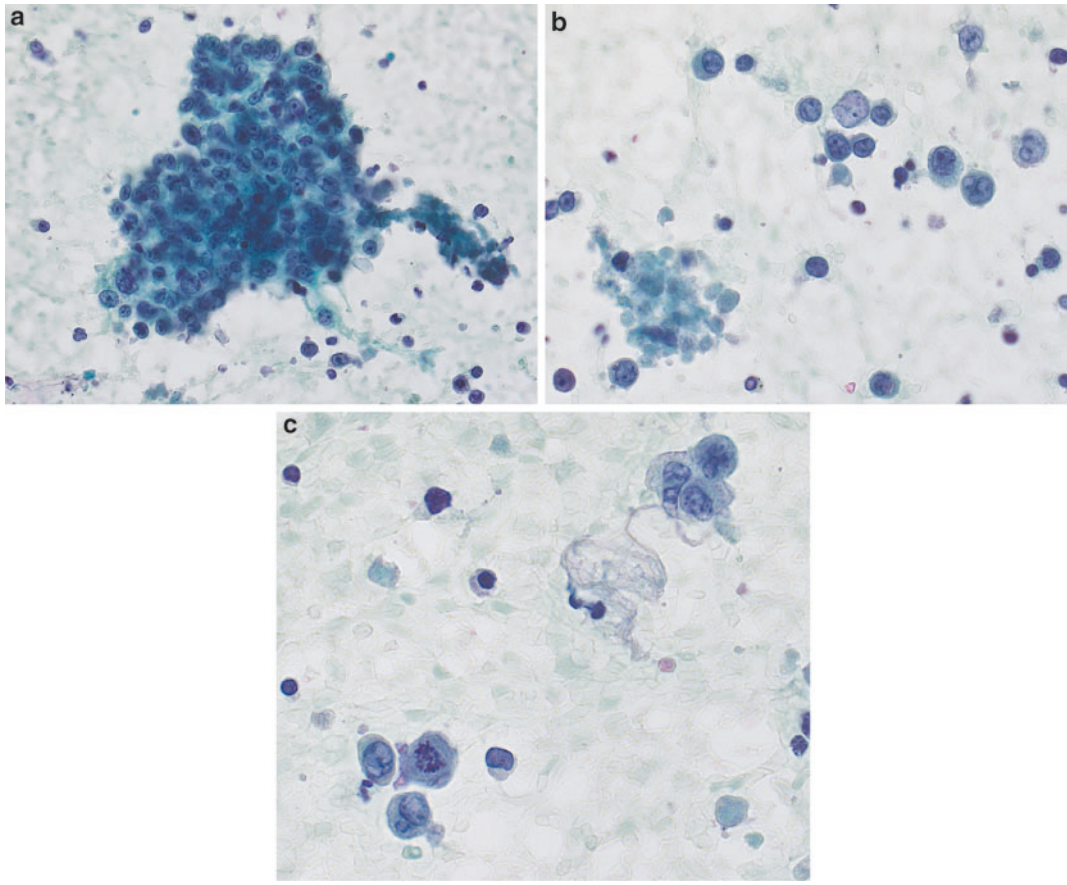
- **Incidence**
  LCNEC account for approximately 3% of all lung cancers.
- **Age**
  The median age is >60 years.
- **Sex**
  They are more common in men.
- **Site**
  Over 80% of LCNEC are peripheral. At time of diagnosis, most present with locoregional lymph node metastases (>70%), and 40% have already metastasized to distant sites.
- **Treatment**
  Treatment of LCNEC is not well established. Retrospective studies suggest that LCNEC respond better to chemotherapy regimens used for small cell lung carcinoma (SCLC), including platinum and etoposide. Early stage LCNEC is surgically resected.
- **Outcome**
  LCNEC have a worse prognosis than other NSCLC, and survival curves are very similar to those observed in SCLC. The prognosis is stage dependent and the 5-year survival rates range from 15% to 57%.

Macroscopy

Most LCNEC present as a large, necrotic tumor in the periphery of the lung.

Microscopy

LCNEC has non-small cell cytological features (Fig. 1). The cells are pleomorphic, intermediate to large in size with a moderate to abundant amount of cytoplasm and sharp cellular borders. The nuclei have coarse or irregular vesicular chromatin and prominent nucleoli. However, in some cases, the nuclei can be hyperchromatic with a nonvesicular, granular chromatin and inconspicuous nucleoli. The cells are arranged in variably sized tight cohesive cell clusters and single cells, often with nuclear molding, typically in a necrotic background. Within the cell clusters, peripheral



Large Cell Neuroendocrine Carcinoma, Cytological Findings, Fig. 1 Large cell neuroendocrine carcinoma (LCNEC) (lung, transbronchial fine-needle aspiration), (**a**) Carcinoma cells arranged in a large cohesive cell cluster. The cells are pleomorphic, with a moderate amount of cytoplasm. The nuclei have coarse and irregular vesicular chromatin and prominent nucleoli (Papanicolaou stain, magnification ×400). (**b**) Cell discohesion and necrosis is typical for LCNEC as seen in this field with single cells and naked nuclei (Papanicolaou stain, magnification ×600). (**c**) Mitoses (left lower corner) are typically present but less frequent compared to histological specimens (Papanicolaou stain, magnification ×600)

nuclear palisading and rosette-like structures can be observed.

Immunophenotype

Diagnosis of LCNEC requires demonstration of neuroendocrine differentiation by immunostains. Like in small cell lung carcinoma (SCLC), the most reliable neuroendocrine marker is CD56, which should be used in a panel together with chromogranin and synaptophysin. At least one of these markers should be positive. Approximately 50% of LCNEC express TTF-1. TTF-1 can be positive in extrapulmonary high-grade neuroendocrine carcinomas and is therefore not specific for a pulmonary primary. Cytokeratin 7 is frequently expressed (>70%). The Ki-67 labeling index is high (>50%).

Molecular Features

Targetable oncogenic driver mutations have rarely been reported in LCNEC, and therefore, testing for EGFR mutations and ALK rearrangements should be performed in advanced stage disease.

Differential Diagnosis

The distinction between LCNEC and NSCLC, especially poorly differentiated adenocarcinoma (LCNEC commonly express TTF-1 and CK7), is difficult. One should keep in mind that 10–20% of NSCLC, adenocarcinomas more commonly than squamous cell carcinomas, can express neuroendocrine markers. The possibility of LCNEC should therefore only be considered in a high-grade NSCLC with morphological features suggestive of neuroendocrine morphology, and only then neuroendocrine markers should be performed. Cell discohesion, naked nuclei, molding, and necrosis are more commonly observed in LCNEC.

The distinction between SCLC and LCNEC is based on the characteristic nuclear and cytoplasmic features. LCNEC typically show non-small cell characteristics with coarse or irregular vesicular chromatin and prominent nucleoli. Rarely, LCNEC can have fine chromatin and inconspicuous nucleoli, but they will usually form larger more cohesive cell clusters and have more abundant cytoplasm with clear cell borders. However, in rare cases, a clear distinction between LCNEC and SCLC is not possible. In this "gray zone," a diagnosis of high-grade neuroendocrine carcinoma should be rendered and the findings discussed in a note. If typical SCLC coexists with findings suggestive of LCNEC, the tumor is classified as combined SCLC.

The high-grade cytology with extensive necrosis and the presence of mitoses usually easily distinguishes LCNEC from carcinoids.

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Lipoma, Cytological Findings

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Synonyms

Benign adipose tumor

Definition

Lipoma is a tumor composed of adipocytes. Some tumors with capillaries, smooth muscle fibers, chondroid, or spindle cells.

Clinical Features

- **Incidence**
  Most common soft tissue tumor.
- **Age**
  All ages are affected but children rarely.
- **Sex**
  No sex predilection.
- **Site**
  Subcutaneous or deep soft tissue. All areas but proximal extremities and trunk dominate.
- **Treatment**
  Surgery.
- **Outcome**
  Intramuscular lipomas have a relatively high recurrence rate.

Microscopy

Lipoma, common variant: adipocytes with large univacuolated cytoplasm and dark eccentric nuclei. Most cells in clusters of varying size. Capillaries seen in most fragments.
Spindle cell variant: adipose tissue of mature type, monomorphic spindle cells both dispersed and in irregular fragments.
Pleomorphic variant: fragments of mature fat spindle cells, large cells with irregular hyperchromatic nuclei and floret cells (Figs. 1–3).

Immunophenotype

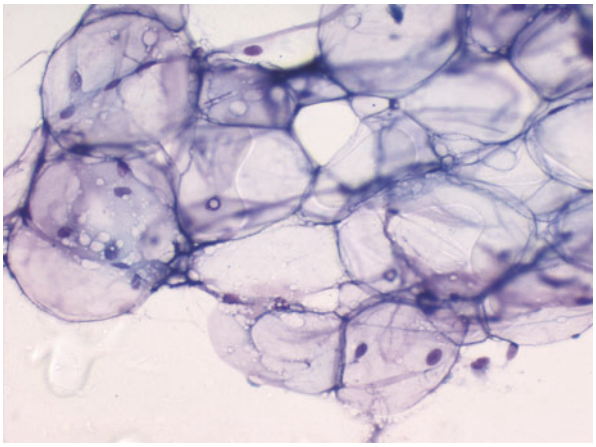
Vimentin, S-100 positive.

Molecular Features

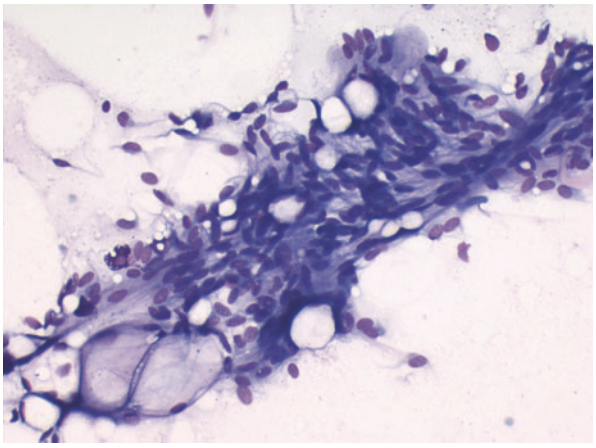
Chromosome aberrations seen in a majority.

Differential Diagnosis

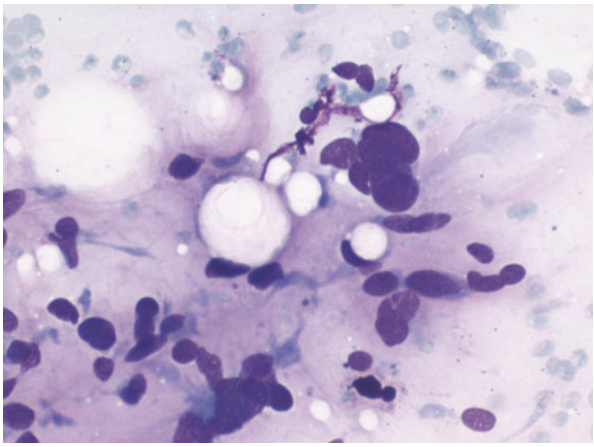
Atypical lipoma.



Lipoma, Cytological Findings, Fig. 1 Lipoma, common variant: mature adipocytes forming an irregular cluster with small oval nuclei. MGG



Lipoma, Cytological Findings, Fig. 2 Lipoma, spindle cell variant: mature adipocytes with numerous bland-looking oval nuclei and poorly preserved spindle-shaped cytoplasm. MGG



Lipoma, Cytological Findings, Fig. 3 Lipoma, pleomorphic variant: cells with hyperchromatic pleomorphic nuclei and poorly preserved cytoplasm. Fat droplets in a slightly myxoid background. MGG

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Liquid-Based Cytology

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Synonyms

LBC; Liquid-based Pap tests

Definition

Liquid-based cytology (LBC) is a state-of-art technology intended to improve the detection of abnormalities in cervical cytology and to reduce the number of false-negative and inadequate cervical samples. LBC involves an altered slide preparation technique, by not making a smear of the material obtained on the spatula/collection device as was done in conventional smears, but placing it in a preservative fluid in order to generate a suspension of cells that is subsequently used to deposit a thin layer of cells in a smaller area on the glass slide. The technique produces uniformly well-fixed cytologic preparations and also reduces contamination by blood, polymorphs, and mucus with minimal cell loss (Zahniser and Sullivan 1996). LBC also increases the feasibility of Human Papilloma Virus (HPV) testing as a screening tool, since it enables an HPV test to be carried out on the same sample used for cytology, and to be restricted to those samples, where the cytology result has proved abnormal (Manos et al. 1999). There are also advantages of LBC in non-gynaec samples like fluids and FNAC (▶ Fine needle aspiration cytology) samples, wherein additional ancillary tests such as immunocytochemistry and molecular testing can be applied to a single sample in diagnostically challenging cases. Conventional cytology lacks precision due to one or more of the following reasons: (i) Pap smears are admixed with mucus/blood, leading to smear reported as inadequate or unsatisfactory. (ii) Multilayering of cells obscures diagnostic fields, leading to both false-negative and sometimes false-positive diagnosis. To a large extent, these drawbacks of conventional smears are overcome by LBC (Table 1). A reduction in inadequate rate of cervical samples (from 9% in conventional cytology to 1–2% in LBC), overall cost, and the backlog of cervical samples was noted in a pilot study on LBC in UK. Also, the sensitivity of detection of high-grade squamous intraepithelial lesion was comparable to the conventional cervical smears (Moss et al. 2004). National Institute for Clinical Excellence (NICE) recommended LBC to be used in National Health Service cervical screening programmes (NHSCSP) in England and Wales as the primary means of processing and screening cervical samples in October 2003.

Liquid-Based Cytology, Table 1 Main differences between conventional smears and liquid-based cytology smears

Conventional smears	Liquid-based smears
Heterogeneous presentation	Homogeneous and uniform presentation
Variable fixation	Uniform fixation
Thick smears with obscuration by mucus, blood, or inflammatory exudate	Thin uniform smears with far less inflammatory exudates, blood, or mucus
Dirty background	Clean background
300–500 k cells/ smear	50–70 k cells/ smear
Nuclear details less clear	Nuclear details more clear
Smear spread over the whole of the slide	A small circular smear
No residual material is left for any additional tests	Residual sample can be used for making additional smears or for HPV & molecular testing

Obtaining the Cervical Sample

LBC Pap test involves the use of cervical brush or a combination of a plastic spatula and endocervical brush to obtain a cervical sample. The inner bristles of brush are inserted into the endocervical canal, and outer bristles remain in contact with the ectocervix (NHS CSP 2006). The brush is rotated inside the endocervical canal, five times in a clockwise direction. A liquid-based cytology sample is obtained by detaching the head of the brush into a vial containing a proprietary transport fluid or by removing after rinsing the brush thoroughly in the preservative vial.

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Liver Metastasis, Cytological Findings

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Synonyms

Secondary liver focus of malignancy

Definition

Liver metastasis are secondary deposits from a primary malignant tumor from another organ that is not directly conected to the liver.

Clinical Features

- **Incidence**
  Liver metastasis is the most frequent malignant tumor encountered in the adults (Centeno 2006). In most of the cases, there is a known history of tumor present, but metastasis from an unknown primary is a crucial problem with which pathologists deal in daily practice. Adenocarcinoma is the most frequent type of carcinoma presenting as unknown primary, accounting for approximately 80%. Squamous cell carcinomas account for another 15%, and metastases of other tumor type's account for the remaining 5% (Centeno 2006).
- **Age and Sex**
  There is no predilection for age or sex in the adult neoplasia.

- **Site**
  Site of origin can be any organ. Colorectal adenocarcinomas are the ones that more frequently metastasize to the liver, but other tumors such as pancreatic, stomach, breast, and lung must be differentiated because of the huge impact on therapeutic options and different outcomes. Neuroendocrine tumors can arise from almost every organ. Metastatic sarcomas to the liver are rare; gastrointestinal stromal tumors and uterine leiomyosarcomas are the most frequent ones (Pitman 2010).
- **Treatment**
  Different treatment modalities other than surgery, chemotherapy, and radiotherapy have been used for metastatic liver disease like radioembolization; microwave, radiofrequency, and ultrasound ablation; and laser-induced interstitial therapy; cryotherapy; and local drug administration such as alcohol injection, endotumoral chemotherapy (Qian 2011).

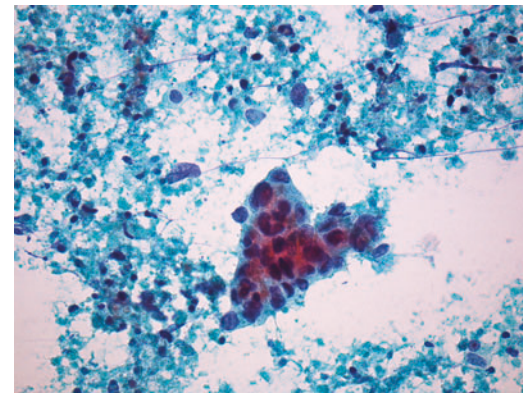
Macroscopy

In general, liver metastases are multiple, but a unique nodule may also be metastatic. Aspirates from colorectal adenocarcinoma are often necrotic and melanoma may have a brownish appearance.

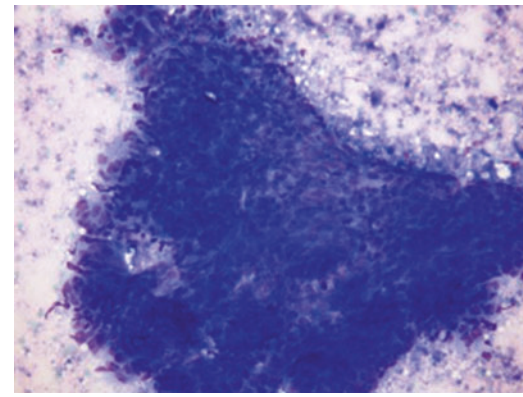
Microscopy

In most of the cases, microscopy reproduces primitive tumor. Lymphomas and small cell tumors are the best examples. The greatest challenge is to differentiate adenocarcinoma of unknown primitive as it changes greatly the therapeutic approach. Some have characteristic features, but often they overlap.

Rapid evaluation of the aspirates will allow splitting the aspirate or repeat the procedure to ancillary techniques like flow cytometry (▶ Flow Cytometry on FNA Material) to diagnose lymphomas (Flow Cytometry, Immunophenotyping) or cell-block and liquid-based cytology. These can be very important steps to reach final diagnosis.



Liver Metastasis, Cytological Findings, Fig. 1 Dirty background, sometimes with mucus. Small groups of cells with prominent nucleoli (Pap stain)

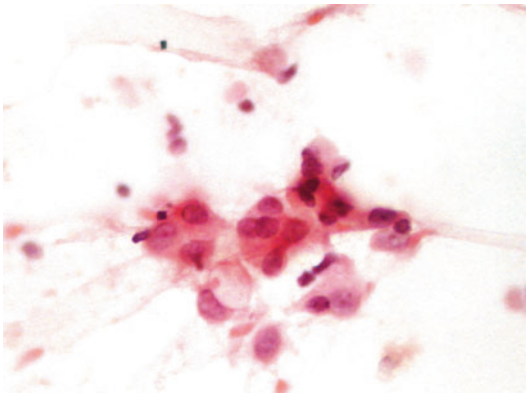


Liver Metastasis, Cytological Findings, Fig. 2 Dirty background with irregular sheets. Note some cigar-shaped nuclei (MGG stain)

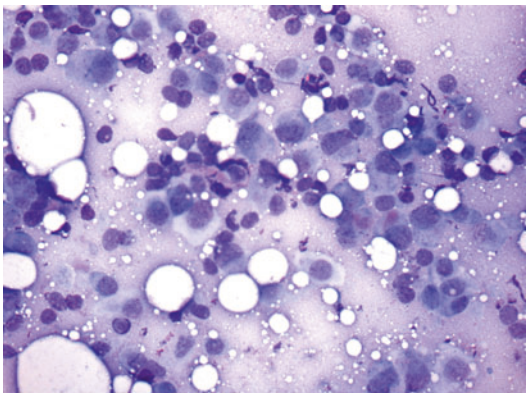
See "▶ Liquid-Based Cytology"

Features of some metastatic tumors are depicted below:

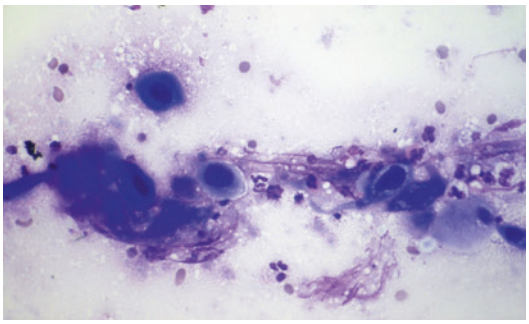
- **Colonic adenocarcinoma** (Figs. 1 and 2)
  Dirty background, sometimes with mucus
  Cigar-shaped nuclei
  Prominent nucleoli, often multiple
  Irregular sheets, sometimes with tubules or acini
  Intracytoplasmic mucin can be detected (Mucicarmin)
- **Breast Carcinoma (Ductal)** (Figs. 3 and 4)
  Monomorphous cell population



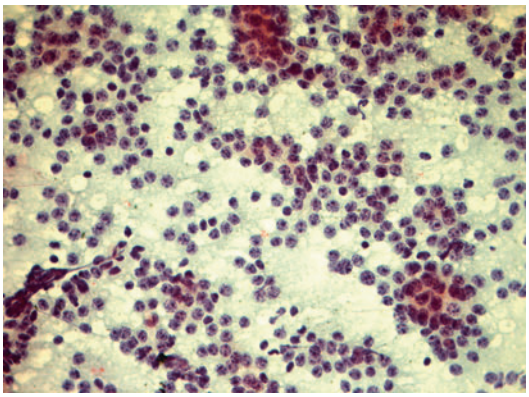
Liver Metastasis, Cytological Findings, Fig. 3 A flat group where intracytoplasmic lumens can be seen (Pap stain)



Liver Metastasis, Cytological Findings, Fig. 4 Monomorphous cell population; some cone-shaped cells (MGG stain)



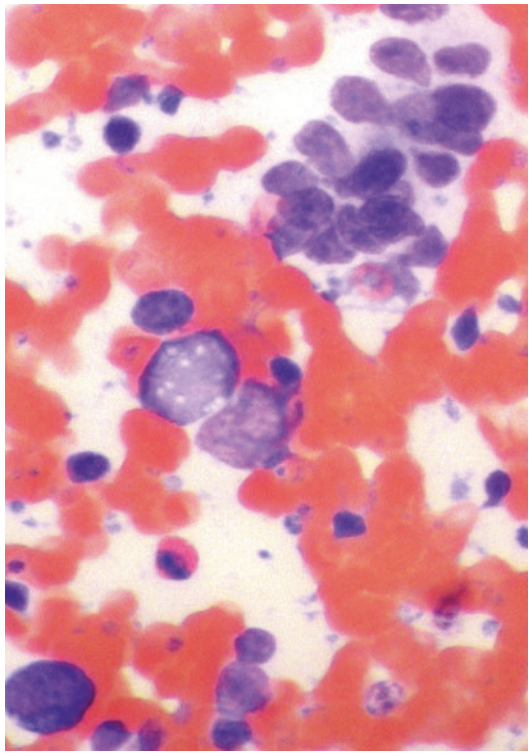
Liver Metastasis, Cytological Findings, Fig. 5 Large hyperchromatic nuclei with irregular nuclear membranes and dense cytoplasm (MGG stain)



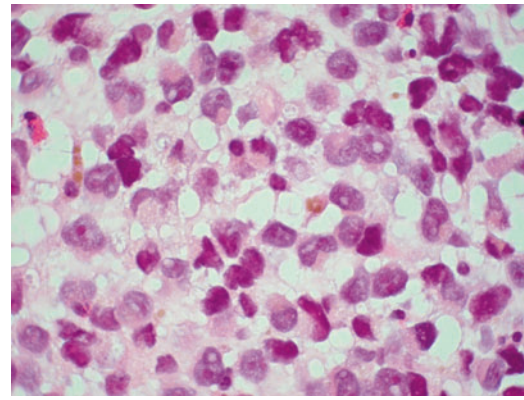
Liver Metastasis, Cytological Findings, Fig. 6 Isolated cells with scant visible cytoplasm, nuclei with coarse chromatin ("salt and pepper" pattern) (Pap stain)

Flat groups
Target cells (intracytoplasmic lumens)
Cone-shaped cells

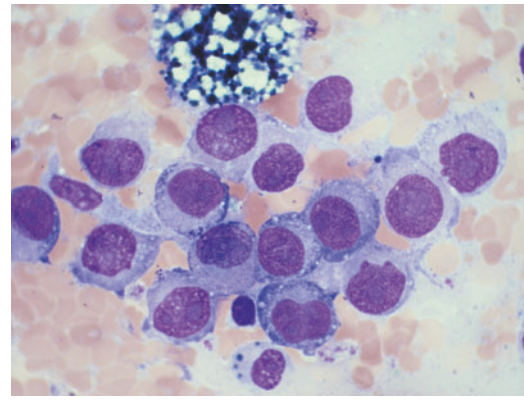
- **Squamous cell carcinoma** (Fig. 5)
  See "► Squamous Cell Carcinoma, Invasive"
  Large polygonal cell in clusters or single
  Large hyperchromatic nuclei with irregular nuclear membranes
  Dense cytoplasm
  Keratinizing cells are orangophilic on Papanicolaou stain
- **Neuroendocrine carcinoma (well differentiated)** (Fig. 6)
  Small and uniform cells with scant visible cytoplasm
  Nuclei with coarse chromatin ("salt and pepper" pattern)
  Isolated cells or forming trabeculae with vascular cores
- ► **Small cell carcinoma**
  See "► Small Cell Carcinoma, Cytological Findings"
  Nuclear and cytoplasm debris in the background
  Small blue cells single or in clusters
  Nuclear molding
  Hyperchromatic and stippled chromatin
  Necrosis and apoptosis
- **Large cell lymphoma** (Fig. 7)
  Lymphoglandular bodies (clumps of stripped cytoplasm) in the background



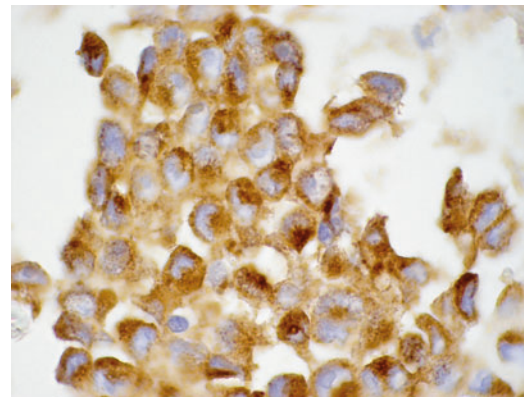
Liver Metastasis, Cytological Findings, Fig. 7 Single cells, here with artifactual aggregation. Note lymphoglandular bodies on the background



Liver Metastasis, Cytological Findings, Fig. 8 Variable cell morphology. Note brown pigment in the cytoplasm (cell-block – H&E)



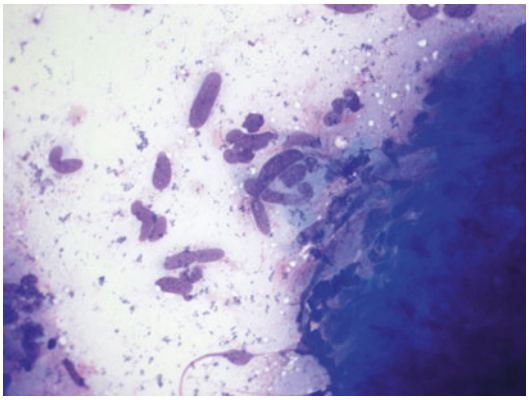
Liver Metastasis, Cytological Findings, Fig. 9 Centrally or eccentric located nuclei ("plasmocytoid" aspect), with large nuclei



Liver Metastasis, Cytological Findings, Fig. 10 Immunohistochemistry with HMB45 (cell-block)

Discohesive, single cells, may have artifactual aggregation
Coarse and peripherally clumped chromatin
Scant cytoplasm but may have large cells (e.g., anaplastic large cell lymphoma)

- **Malignant melanoma** (Figs. 8–10)
  See "▶ Melanoma, Cytological Findings"
  Variable cell morphology: generally polygonal and large, may be spindle or small blue cell.
  Eccentric nuclei (plasmacytoid) or central with large nucleoli and frequently intranuclear inclusion.
  Cytoplasm frequently not pigmented, nongranular.
  Histochemistry with Fontana–Masson will stain melanin black but also stains lipofuscin present in hepatocytes.
  Immunohistochemistry with HMB45, S100 and Melan-A can be used to support the diagnosis.



Liver Metastasis, Cytological Findings, Fig. 11 Spindle cells with delicate cytoplasm around both ends of the nucleus (MGG stain)

- **Gastrointestinal stromal tumor (GIST)** (Fig. 11)
  Spindle cells, usually monomorphic but can have epithelioid features.
  Delicate cytoplasm around both ends of the nucleus.
  Relatively bland nuclei.
  Little artifact.
  Immunohistochemistry with CD117 is highly specific but is not always present.

Differential Diagnosis

Clinical data are crucial for the correct interpretation of cytomorphology. It is also important to guide immunohistochemical approach, when morphology is not enough. Cholangiocarcinoma is difficult to distinguish from metastatic adenocarcinoma. It expresses cytokeratins 7 and 19 but not cytokeratin 20.

See "▶ Cholangiocarcinoma, Cytological Findings"

Cytokeratin 7 and 20 expressions can discriminate metastatic adenocarcinoma, with high positive predictive value. Probability for colorectal carcinoma metastasis when $CK7^{-}/CK20^{+}$ is equal to 78% (Centeno 2006).

Panels of several antibodies have been studied to discriminate site of origin. Here are some useful immunohistochemical markers (Table 1):

Liver Metastasis, Cytological Findings, Table 1 List of some useful immunohistochemistry antibodies to determine primary tumor

CD117 (c-kit) and CD34	GIST
CEA	Cytoplasmic staining in adenocarcinomas
Desmin and Actin	Leiomyosarcoma
GCDFP15	Breast
HMB45, S100,	Melanoma,
Inibin	Adrenocortical carcinoma
Melan-A	Adrenocortical carcinoma, melanoma
Estrogen receptors	Breast, ovarian, endometrial
PAX 8	Renal cell carcinoma
PSA	Prostate
Synaptophysin, neuron-specific enolase, and chromogranin	Neuroendocrine carcinomas
	GI: $CDX2^{+}$
	Pulmonary: $TTF1^{+}$
TTF1	Non-mucinous pulmonary adenocarcinomas; thyroid carcinomas
Uroplakin	Urothelial carcinoma
WT1	Ovarian serous tumors

Based on Bosman et al. (2010)

Much more difficult is to distinguish cholangiocarcinoma from metastatic adenocarcinoma. They express cytokeratins 7 and 19 but not cytokeratin 20.

See "▶ Cholangiocarcinoma, Cytological Findings"

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Lobular Breast Carcinoma, Cytological Findings

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Synonyms

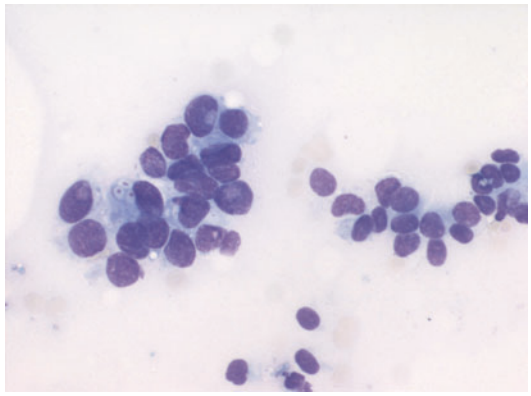
Invasive lobular carcinoma

Definition

Invasive breast carcinoma composed of isolated cells, arranged in a single-file linear pattern in a fibrous stroma (Fig. 1).

Clinical Features

- **Incidence**
  This carcinoma represents 2–15% of all breast carcinoma with a high rate of bilaterality (5–20%).



Lobular Breast Carcinoma, Cytological Findings, Fig. 1 Invasive lobular carcinoma. Note the cell molding and presence of intracytoplasmic lumina (Giemsa staining) (Courtesy of Dr. Edneia Tani)

- **Age**
  It can occur at any age but it is rare in patients younger than 25 y/o and older than 80 y/o with a peak incidence at 57–65 y/o.
- **Sex**
  It is 200 times more frequent in women than men.
- **Site**
  Any part of the breast, been slightly more frequent in central localization than ductal carcinoma.
- **Treatment**
  Surgery, radiotherapy, chemotherapy, and hormonotherapy.
- **Outcome**
  This cancer shows a lymphatic and hematological dissemination. Central nervous metastases occur as carcinomatous meningitis with a typical leptomeningeal infiltration. The abdominal metastases involve the serosal surfaces, retroperitoneum, or ovary. The important factors of prognosis are represented by primary tumor size and nodal status. Some studies have shown a much more frequent local recurrence in the breast after 5 years in lobular carcinomas than in ductal cancers.

Macroscopy

Due to its infiltrative nature characterized by single cells, invasive lobular carcinomas present as irregular and poorly delimited tumors.

Microscopy

The cytological specimen is defined by a hypocellular and monomorphic pattern of cells with medium size and organized as single cells or small aggregates sometimes in a linear behavior. The cells are small or medium with a higher N/C ratio showing granular chromatin and small nucleoli. The key features for the cytological diagnosis are (a) low cellularity of the specimen, (b) presence of single cells and small clusters, (c) uniform small-medium cell with higher N/C ratio, (d) evidence of signet ring cells, and (e)

absence of bipolar nuclei. In the pleomorphic subtype the cells are isolated with nuclear pleomorphism. These cases on cytology cannot be differentiated from ductal carcinomas.

Immunophenotype

Lobular carcinomas are typically identified by the loss of E-cadherin expression in contrast with ductal carcinoma. Lobular carcinomas are invariably ER and PR positive. HER2 amplification and overexpression are rare and more frequent in the pleomorphic variant.

Molecular Features

E-cadherin inactivation is the most common genetic alteration in lobular carcinomas. Somatic truncating mutations in the E-cadherin gene (CDH1) together with LOH and promoter methylation contribute to the loss of expression. Most of lobular carcinomas are luminal A molecular subtype. Loss of 16q and gain on 1q and 16p are frequent in lobular carcinomas. In the pleomorphic variant amplifications at 8q24, 17q12, and 20q13 are additional characteristics.

Differential Diagnosis

Differential diagnosis on cytology is extremely difficult especially its differentiation from the ductal cell carcinoma. An underdiagnosis might be done in presence of histiocytoid changes which can be misdiagnosed as benign histiocytes.

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Low-Grade Papillary Urothelial Carcinoma, Cytological Findings

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Synonyms

Transitional cell carcinoma (term not recommended)

Definition

Historically, urothelial tumors were classified into the three histological groups, grade 1, grade 2, and grade 3, that largely matched with the three-tiered cytological grading systems commonly used and also proposed by L.G. Koss. In 1998, the World Health Organization and International Society of Urologic Pathologists (WHO/ISUP) established a new histological classification system for urothelial neoplasm that was incorporated in the 2004 WHO "Blue Book." As opposed to the previous WHO classification from 1973, the three-tiered grading system was simplified to a low-grade and high-grade group, with the low-grade group almost exclusively being diagnosed in noninvasive neoplasia including pTa tumors and dysplasia (=low-grade intraurothelial neoplasia). Cytology from voided urine or bladder washing specimens has a high sensitivity in high-grade UC but low sensitivity in low-grade UC (LGUC). Therefore, negative cytology does not exclude the presence of a tumor. The 2004 WHO classification introduced a new lesion called papillary urothelial neoplasia of low malignant

potential (PUNLMP) in order to avoid the term carcinoma for this lesion that has a negligible progression risk. By definition, PUNLMP lacks cytological atypia and can therefore not be diagnosed by cytology. Given the generally good prognosis of LGUC, the low sensitivity of cytology in these tumors is acceptable and does no question the value of cytology as a means to detect the more important high-grade lesions. On the other hand, diagnosis of a LGUC in a bladder washing at the time of a papillary tumor that is visible at endoscopy is an academic exercise with no practical value – the value of cytology lies in excluding simultaneous high-grade lesions (e.g., carcinoma in situ) that may not be detectable by histology. Since the diagnosis of LGUC by cytology is not critical from a clinical point of view, it is given low priority in the forthcoming "Paris system of reporting urinary cytology." Nevertheless, in situations, in which the diagnosis can be made with certainty (mainly in bladder washings from bladders with visible tumors), cytologists should make the call and not hide behind an unspecified diagnosis of uncertainty.

Clinical Features

- **Incidence**
  Low-grade noninvasive papillary urothelial carcinomas make the majority of all urothelial carcinomas (approximately 70%).
- **Age**
  Urothelial tumors are typically a disease of elderly people. The median ages of patients at the time of the initial diagnosis are 69 years in men and 71 years in women.
- **Sex**
  Urothelial carcinoma is more common in male than in female. Smoking increases the risk of bladder cancer by a factor of 2–6.
- **Site**
  Urothelial carcinomas are often multifocal due to a cancer field effect of the whole bladder and upper urinary tract or due to intraepithelial spreading of tumor cells. Approximately 5–10% of all recurrences occur in the upper urinary tract.
- **Treatment**
  Noninvasive, low-grade papillary urothelial carcinomas tumors are treated with transurethral resection followed by lifelong surveillance for detection of recurrences.
- **Outcome**
  LGUC have a recurrence rate of low-grade UC (50–70%) but do only rarely progress to life-threatening muscle-invasive bladder cancer.

Macroscopy

The procedures for obtaining and processing voided urines and bladder washings are described in detail elsewhere in this Encyclopedia ("▶ Bladder Washing Specimen" and "▶ Urine Specimen, Cytology").

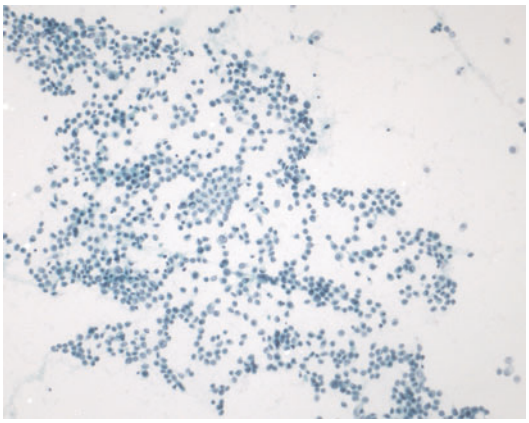
Microscopy

It is a common understanding that cytological diagnosis of low-grade urothelial lesions is difficult if not impossible in most cases. This is in particular true for voided urines, where subtle morphological details are often obscured by degenerative changes. In addition, these low-grade neoplastic lesions do not shed as easily as high-grade lesions. The fact that most of the low-grade lesions are chromosomally stable and hence diploid explains why the abnormalities of the nuclear chromatin – the most important diagnostic feature for the diagnosis of urothelial carcinoma – are barely present or minimal. Cytologist does not need to worry about PUNLMP lesions that are histologically defined by thickened urothelium with intact polarity and are composed of cells that show nuclear enlargement but no membrane irregularities or chromatin coarseness – they cannot be diagnosed cytologically by definition. In bladder washings, a definitive cytological diagnosis of LGUC is often possible, although this is mainly restricted to patients with visible tumors but much less common in the rare patients under surveillance with isolated dysplasia (=low-grade intraurothelial neoplasia). Most LGUC diagnosed

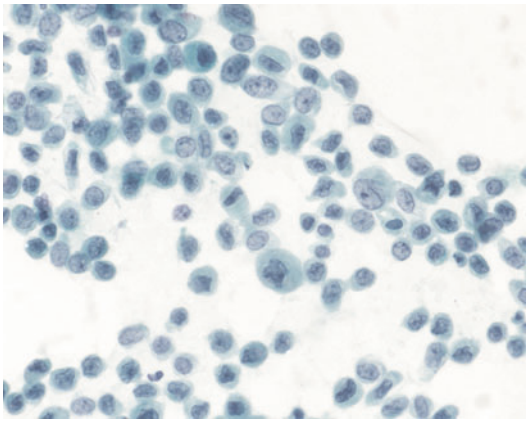
by cytology correspond to the low-grade end of the former G2 category. At low magnification, LGUC in bladder washings presents as a monotonous picture of dyscohesive cells lacking the heterogeneity of reactive benign and reactive bladders. Presence of pseudopapillary fronds or three-dimensional urothelial cell clusters is a rather common finding in normal bladder washings and often over-interpreted as papillary low-grade tumors. At higher magnification, LGUC cells have an increased nuclear/cytoplasmic ratio and eccentric nuclei with somewhat irregular contours, yet in general a uniform granular chromatin and only discrete nuclear membrane irregularities and thickened nuclear membranes (Figs. 1, 2, 3, and 4). The cytoplasm appears homogeneous and less open as in reactive urothelial cells. Umbrella cells are variably present. Among the previous G2 category, one can recognize two variants defined by different qualities of the nuclei. One type has rather bright and vesicular nuclei with few indentions, an open chromatin, but discrete irregularities of the thickened nuclear membrane – these cells belong almost invariably to the LGUC (Fig. 3). The other G2 variant contains dark and hyperchromatic nuclei with coarse chromatin but lacks the clear polymorphy of G3 cells (Fig. 5). Part of this G2 variant is difficult to classify into low versus high grade and qualifies for the AUC-H category ("atypical urothelial cells suspicious for high-grade urothelial carcinoma").

Immunophenotype

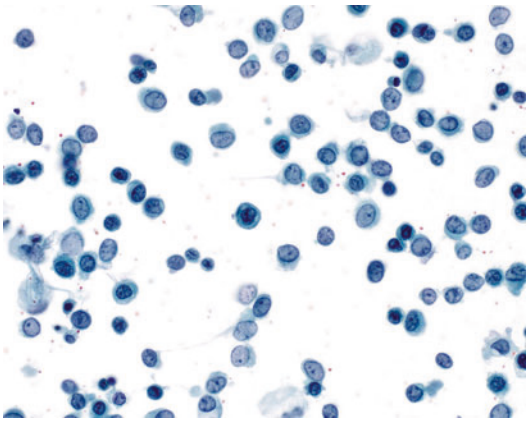
Urothelial cells are diffusely positive for cytokeratin 7, p63, and GATA-3. Cytokeratin 20,



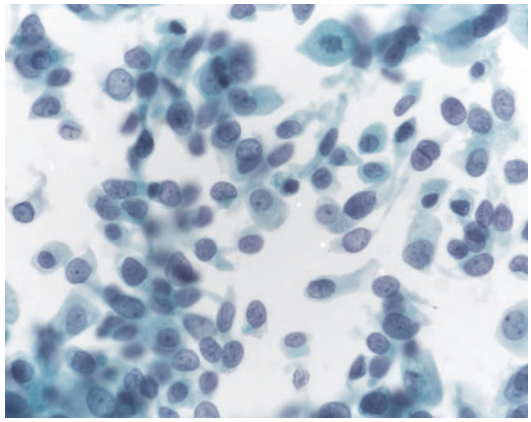
Low-Grade Papillary Urothelial Carcinoma, Cytological Findings, Fig. 1 Low-grade urothelial carcinoma. At low magnification, there is a monotonous picture of one population of urothelial cells (bladder washing, Papanicolaou, ×100)



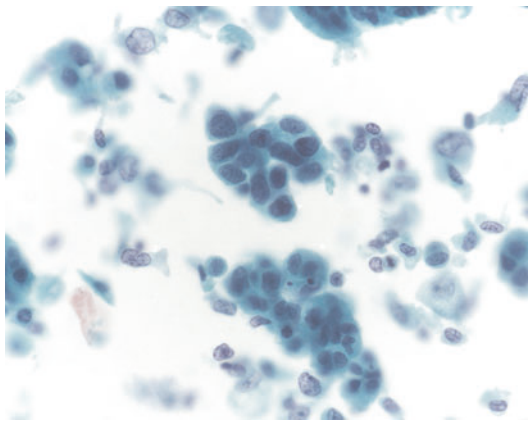
Low-Grade Papillary Urothelial Carcinoma, Cytological Findings, Fig. 2 Low-grade urothelial carcinoma. At higher magnification, the nuclei appear slightly enlarged and eccentric, and they have discrete irregularities of the nuclear contour and a slight thickening of the nuclear membrane. The chromatin is open (bladder washing, Papanicolaou ×400)



Low-Grade Papillary Urothelial Carcinoma, Cytological Findings, Fig. 3 Low-grade urothelial carcinoma. Atypical urothelial cells with enlarged, vesicular nuclei that have a mostly clear chromatin but still some degree of coarse granularity. The N-C ratio is increased. (bladder washing, Papanicolaou, ×400)



Low-Grade Papillary Urothelial Carcinoma, Cytological Findings, Fig. 4 Low-grade urothelial carcinoma. There is a population of urothelial cells with hyperchromatic, eccentric nuclei with mild irregularities of the contour and the nuclear membrane. The N-C ratio is increased. However, the alterations did not reach the level of HGUC (bladder washing, Papanicolaou, ×400)



Low-Grade Papillary Urothelial Carcinoma, Cytological Findings, Fig. 5 Borderline cytology between LGUC and HGUC, consistent with atypical urothelial cells suspicious of high-grade urothelial carcinoma (*AUC-H*). The nuclear alterations are more pronounced as in Fig. 4 including polymorphy, hyperchromasy, and irregularities of the nuclear membrane (bladder washing, ×400)

which mainly stains normal umbrella cells in the benign urothelium, becomes often diffusely positive in urothelial carcinoma. Therefore, CK20 immunocytochemistry has been proposed as a potential diagnostic marker in urinary cytology. LGUC including carcinoma may reveal weak physiological positivity for p53 on the basal layers but lack the abnormal and strong p53 positivity often seen in high-grade UC due to p53 mutation. None of these markers are currently recommended for routine use in urinary cytology.

Molecular Features

Loss of 9p21 (site of p16) is a common finding in LGUC, as increased chromosome copy numbers also occur. Thus, it is not surprising that UroVysion® multi-target fluorescence in situ hybridization (FISH) assay (Abbott Molecular Inc., Des Plaines, IL) for detecting polysomies of chromosomes 3, 7, and 17 and deletion of 9p21 increases the sensitivity of cytology for the diagnosis of LGUC. However, given the low risk of progression of LGUC, it is rarely needed to enforce the diagnosis with ancillary techniques. Also other molecular screening tests are currently not recommended for routine use in patients with suspicion of UC or under surveillance, although there is evidence that FISH and other tests such as ImmunoCyt®/uCyt+ (Scimedx, Denville, NJ, USA) in patients under surveillance might help to stratify the risk of recurrence and to reduce control cystoscopy in patients at low risk. FISH can also be helpful in specimens of the upper urinary tract, which is not easily accessible by endoscopy.

Differential Diagnosis

Reactive changes of benign urothelial cells are the main differential diagnosis. In particular, three-dimensional cell groups and clusters that may have a papilliform appearance are often misinterpreted as evidence of low-grade papillary urothelial tumors, but are in fact common in instrumented urinary specimens such as bladder washings and preparations of the upper urinary tract.

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Lung Metastasis, Cytological Findings

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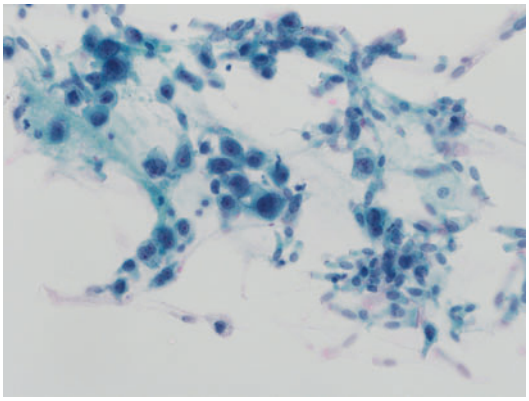
Definition

Spread of malignant neoplasm originating from another organ to the lung via vascular/lymphatic channels.

Clinical Features

- **Incidence**
  Pulmonary metastases are often found in advanced stages of carcinoma originating from a number of sites. Metastases in the lung can be found in 30–50% of patients dying from malignant disease. Tumors from the following sites metastasize frequently to the lung: kidney, colorectum, pancreas, breast (see Fig. 1), stomach, and oropharynx.

  Other rare tumors which spread preferentially to the lungs are chorionic carcinoma, osteosarcoma, soft tissue sarcoma, testicular tumors, Ewing sarcoma, and thyroid carcinoma. Primary lung cancer itself can seed metastases in the lung.



Lung Metastasis, Cytological Findings, Fig. 1 Breast cancer: the cells of this metastasizing breast cancer form loose clusters. The nuclei show slight abnormalities with irregular nuclear membranes and prominent nucleoli (bronchial brush, Papanicolaou, 40×)

- **Age**
  The age depends on the age distribution of the primary tumor.
- **Sex**
  The sex depends on the origin of the primary tumor.
- **Site**
  The peripheral middle and lower parts of the lungs are more commonly affected.
- **Treatment**
  The treatment depends on the origin of the primary tumor as well as on the extent of the metastatic disease. Surgical resection is a therapeutic option in single/few metastases esp. in colorectal cancer and sarcomas. Otherwise, systemic treatment is a possibility.
- **Outcome**
  The prognosis of patients with lung metastases, who do not receive treatment, is very poor (<5% 5-year survival). The 5-year survival rates after metastasectomy range from 29% to 52% for soft tissue sarcoma and 79% to 94% for non-seminomatous germ cell tumors.

Macroscopy

Most often, lung metastases are found in the form of multiple nodules in the lung parenchyma (80%). Less often, a solitary mass lesion in the parenchyma (20%) or endobronchial lesions (up

to 20%) may occur. A diffuse lymphangiosis carcinomatosa may be present. In about a quarter of patients with lung involvement of a metastatic tumor, tumor embolisms can be encountered.

Microscopy

Cells of metastatic lesions may be encountered in different cytologic specimen types: sputum, bronchial washing/brush cytology, bronchoalveolar lavage, and fine needle aspirations. It is often not possible to differentiate between primary carcinoma of the lung and a metastatic lesion. Obviously, the clinical history may help to consider a metastasis. In addition, an unusual morphology should raise the suspicion and prompt further assessment, esp. immunocytochemistry or further clinical workup.

Tumors of the gastrointestinal tract may show mucin production and/or signet ring cells. Colonic cancer metastases appear often in clusters. The cells are often columnar with vesicular, enlarged, pleomorphic nuclei and irregular nuclear membranes. A diathesis is common.

In metastases of gastric cancer, numerous signet ring cells may be present. The cells are arranged singly or in small groups.

Metastases of invasive carcinoma of the breast may show aggregates or single cells, often with discrete signs of cellular/nuclear atypia. Intracytoplasmic lumina (ICL) may be encountered (see Fig. 1).

In clear cell carcinoma of the kidney, the tumor cells can form papillary groups or can be attached to extracellular basement membrane-like material. The cells are usually large, with foamy cytoplasm.

Sarcomas can appear in various forms: spindle cell variants, pleomorphic appearances, and as "small round blue cell" lesions.

Melanomas have always to be considered in metastatic lesions of unknown primary in the lung. They may mimic any other tumor entity. Pigmented cells containing melanin are an important clue, but not always present.

Metastases of head and neck cancer are mostly squamous cell carcinomas, which obviously have to be distinguished from primary squamous cell carcinoma of the lung. A high amount of keratinization suggests a primary head and neck cancer rather than a primary lung tumor.

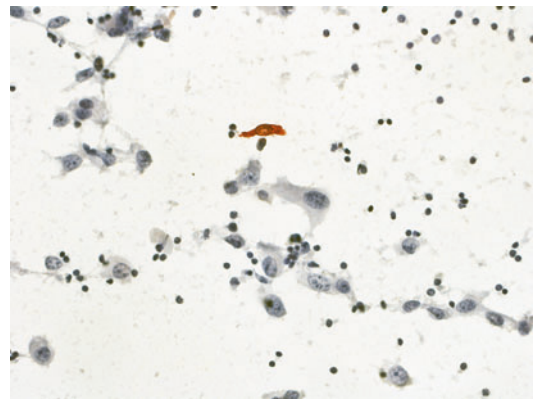
Immunophenotype

In order to distinguish a primary adenocarcinoma of the lung from metastatic adenocarcinoma, a panel of markers may be helpful. TTF1 positivity strongly suggests a lung adenocarcinoma; however, a thyroid tumor has to be considered as well. Helpful for the identification of breast cancer are GATA3, GCDFP, and mammaglobin (in decreasing order) as well as estrogen and progesterone receptors.

CK7+/CK20- tumors may be primary lung adenocarcinoma; however, gastric cancer can display this phenotype as well, although they may be CK+/CK20+. Colonic cancer is commonly CK7-/CK20+/CDX2+. Clear cell carcinomas of the kidney are usually positive for vimentin, pancytokeratin, and CD10 (see Fig. 2).

Molecular Features

Molecular profiling can be used for the identification of the origin in metastases of an unknown primary tumor. Several commercial tests are



Lung Metastasis, Cytological Findings, Fig. 2 Clear cell carcinoma of the kidney: the cells of this clear cell carcinoma of the kidney, which metastasized to the lung, have abundant cytoplasm. The tumor cells are negative for CK7, whereas a ciliated bronchial cell is strongly positive for this marker, therefore serving as an internal control (FNA of a lung lesion, immunocytochemistry for CK7, 40×)

available, which basically compare the RNA profile of the metastases to the profiles of primary tumors.

Differential Diagnosis

The differential diagnoses for metastases are primary carcinomas of the lung and benign lesions of the lung.

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M

Malakoplakia, Cytological Findings

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Definition

Malakoplakia is a rare chronic inflammatory disorder. It is probably caused by the incomplete digestion of bacteria (mostly *E. coli*) in histiocytes due to chronic infection or immunosuppression.

Clinical Features

- **Incidence**
  The disorder is very rare.
- **Age**
  Most patients are older than 50 years.
- **Sex**
  There is a female predominance (female/male ratio: 4/1) in cases which affect the genitourinary tract. At other sites, this disease shows no predilection for sex.
- **Site**
  The urinary bladder is most commonly affected. Other sites in the urogenital tract may be affected. Involvement of other organs (gastrointestinal tract, lung, bone, etc.) is possible.
- **Treatment**
  Antibiotic treatment is administered, if an underlying infection (usually *E. coli*) is found.
- **Outcome**
  The outcome depends on the extent of the disease and the underlying disorder.

Macroscopy

Multiple, soft yellow or brown nodules or polyps ("plaques") are usually found on the surface of the mucosa of the urinary bladder. In other sites of the body, tumorlike soft lesions can be noted.

Microscopy

Histologically, the plaques consist of dense aggregates of histiocytes with granular eosinophilic cytoplasm. These aggregates are most commonly located in the superficial lamina propria of the urinary bladder. So-called Michaelis-Gutmann bodies are characteristically found in the cytoplasm of the histiocytes. These PAS (periodic acidic-Schiff)-positive Michaelis-Gutmann bodies consist of a crystalline core which is surrounded by a homogenous zone. Their size ranges from 5 to 10 μm. They can be highlighted by von Kossa stain and Perls' Prussian blue stain which react with calcium and iron, respectively.

In cytology, large histiocytes with abundant foamy cytoplasm containing Michaelis-Gutmann bodies can be found. However, the diagnosis of malakoplakia by fine needle aspiration is very rare.

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4

Immunophenotype

Macrophages are positive for CD68 positive and negative for CD1a, S-100, and cytokeratines.

Differential Diagnosis

Langerhans cell histiocytosis and xanthogranulomatous inflammation are the main differential diagnosis.

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Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings

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Description

Malignant melanoma. The cytological diagnosis of melanoma is rarely requested. Cytological features and diagnostic criteria are previously reported. Pigmentation of the eyelid margins, occurring in association with conjunctival melanoma, is an ominous clinical sign.

Cross-References

▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
▶ Conjunctiva Cytology, General Aspects
▶ Conjunctival Inflammatory Lesions, Cytological Findings
▶ Conjunctival Lymphoma, Cytological Findings
▶ Conjunctival Melanocytic Tumors, Cytological Findings
▶ Conjunctival Papilloma, Cytological Findings
▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
▶ Cornea Cytology
▶ Cytology of the Orbit and Ocular Adnexa
▶ Eyelids Cytology, General Aspects
▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Meningioma, Cytological Findings
▶ Orbit Cytology, General Aspects
▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Mediastinal Tumor, Fine Needle Aspiration

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Definition

Fine needle aspirates of the mediastinum refer to aspirates targeting lesions occurring in the mediastinal space which is the partition in the middle of the thorax situated between the two pleural sacs and extending from the sternum interiorly to the vertebral column in the posterior. The mediastinal space encompasses all the thoracic viscera except for the lungs and pleura. Because of the wide range of structures within the mediastinum from organs to vessels and nerves, the number of lesions encountered is enormous.

A variety of tumors may be encountered in the mediastinum. Often, these tumors can be accurately diagnosed via minimally invasive ultrasound-guided transbronchial (EBUS) or transesophageal (EUS) fine needle aspiration biopsy. In addition, in recent years, EBUS/EUS-guided FNA has emerged as a safe and effective technique for mediastinal lymph node staging in patients with lung cancer. While mediastinoscopy has been and remains the gold standard technique for sampling mediastinal lymph nodes, EBUS-guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a convenient, efficient, and accurate means of mediastinal lymph node staging. Furthermore, it has been shown that EBUS can improve the diagnostic yield for sampling mediastinal and hilar lymph nodes when compared with other procedures, such as mediastinoscopy, conventional TBNA, and EUS-guided transesophageal FNA.

Recent advances in technology, including real-time ultrasound visualization and navigational bronchoscopy, have contributed to the increased use of EBUS-TBNA. Also, unlike mediastinoscopy, EBUS-TBNA does not require general anesthesia, and compared to mediastinoscopy, EBUS-TBNA is less invasive. In addition, mediastinoscopy is performed in only 27% of patients undergoing lung cancer resection surgery, and lymph nodes are obtained in less than 50% of these procedures. While conventional TBNA is also safer and less expensive than mediastinoscopy, it is "blind" procedure, and as such, it is generally used only to sample bulky subcarinal (station 7) or right lower paratracheal (4R) lymph nodes. Transesophageal EUS-FNA can reach left lower paratracheal (4L) and subcarinal (7) lymph nodes, and it is particularly useful for sampling station 8 nodes and the left adrenal gland.

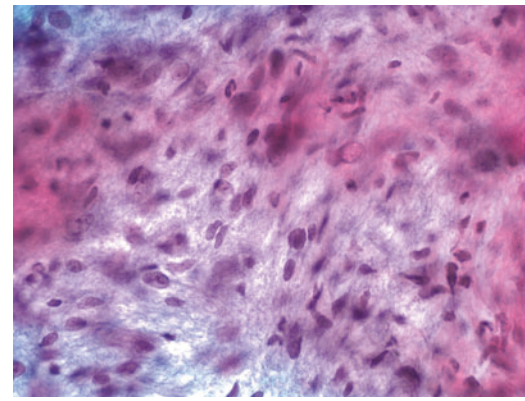
Several types of specimens can be obtained with EBUS guidance, including needle aspiration,

brushing, washing, lavage, and core needle biopsy. Direct smears can be made from needle aspiration and brushing specimens to allow on-site evaluation (Feller-Kopman et al. 2009). The use of on-site evaluation can be of great value in terms of maximizing the diagnostic yield for EBUS-guided procedures. This is likely another factor contributing to the increased application of EBUS-TBNA.

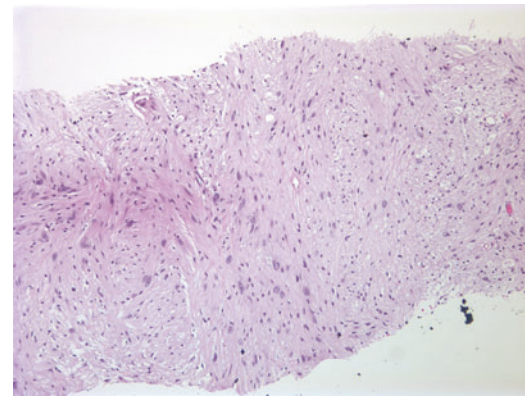
In adults, the most common mediastinal tumors are metastases. Often, these are of pulmonary origin, including small cell carcinoma, squamous cell carcinoma, and adenocarcinomas (cytologic features are similar to those described in lung metastasis, cytology). However, almost any malignant tumor from any organ system can metastasize to mediastinal lymph nodes (Cibas and Ducatman 2009; Demay 2012; Koss and Melamed 2005).

Although rare, the most common primary anterior mediastinal tumor in adults is thymoma (cytologic features described under thymoma, cytology of).

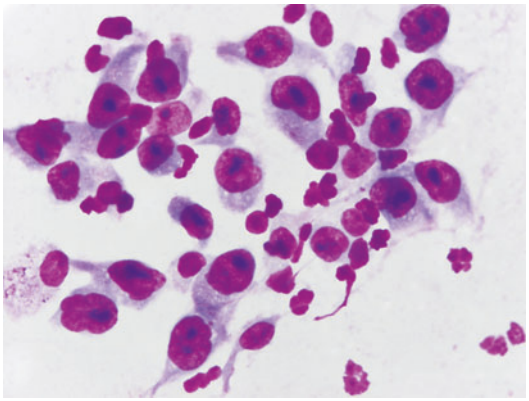
The posterior mediastinum is the most common site of neurogenic tumors. *Schwannomas* are the most common mediastinal neurogenic tumors. Aspirates from schwannomas are highly variable with regard to cellularity (Figs. 1 and 2). Smears should contain cohesive clusters and syncytial sheets of spindle cells with wavy elongated nuclei, most with tapered ends. Nucleoli are inconspicuous. Nuclear atypia is generally minimal, but may be severe in ancient schwannomas. However, even with significant nuclear pleomorphism, a malignant diagnosis should be avoided in the absence of mitotic figures and necrosis. Immunohistochemical reactivity with S-100 should be strong and diffuse in schwannomas. Mediastinal *neurofibromas* usually occur in association with Neurofibromatosis, type 1 (NF-1), and these tumors – unlike elsewhere in the body – are often encapsulated. Smears are generally paucicellular and show loosely organized clusters of spindle cells with elongated wavy nuclei embedded in myxoid stroma. Immunohistochemical reactivity with S-100 should be weak and focal in neurofibromas. The cytologic features of *malignant peripheral nerve sheath tumors* are highly variable. The malignant cells may be epithelioid or spindled, or assume other cytomorphologies. Nuclear pleomorphism is variable, as is the presence of nucleoli. Necrosis and mitoses are often seen (Figs. 3–6). As with neurofibromas, there is usually an association with NF-1, and immunohistochemical reactivity with S-100 should be weak and focal (Klijanienko et al. 2002).



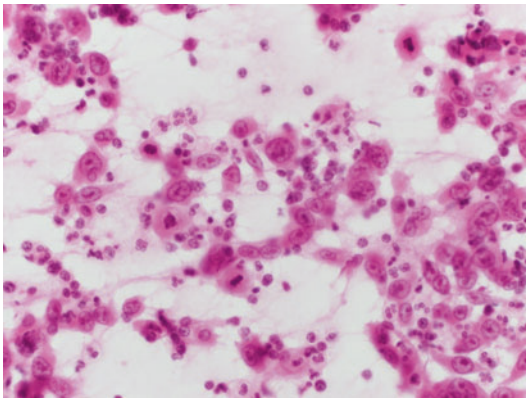
Mediastinal Tumor, Fine Needle Aspiration, Fig. 1 Medium power Papanicolaou stain demonstrating an FNA of schwannoma presenting as a syncytial fragment of fusiform cells with elongated, tapered, and occasionally wavy nuclei in a fibrillar stroma. Notice the peripheral cellular area surrounding a central hypocellular focus (Pap stain)



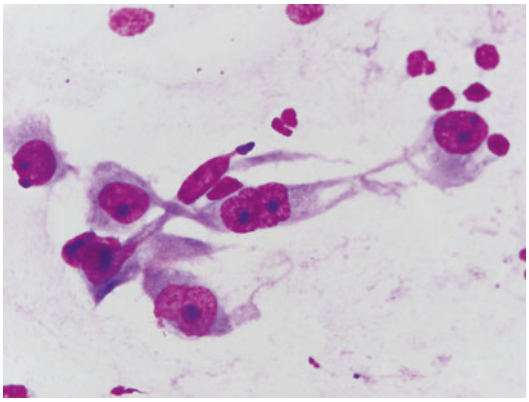
Mediastinal Tumor, Fine Needle Aspiration, Fig. 2 Low power H&E stain demonstrating the correlating core biopsy of schwannoma illustrating the alternating Antoni A (cellular) and Antoni B (hypocellular) areas (H&E stain)



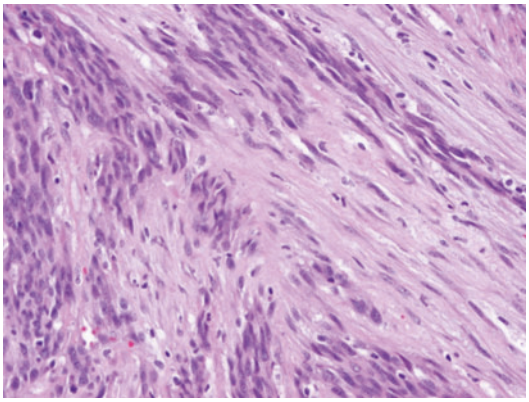
Mediastinal Tumor, Fine Needle Aspiration, Fig. 3 Low power Diff-Quik stain demonstrating an FNA of malignant peripheral nerve sheath tumor showing polygonal to spindled highly pleomorphic nuclei with prominent nucleoli (Diff-Quik stain)



Mediastinal Tumor, Fine Needle Aspiration, Fig. 5 Medium power H&E stain of the same tumor demonstrating the atypical polygonal to spindle cells with loss of cohesion, mitotic and apoptotic figures, and necrotic background (H&E stain)



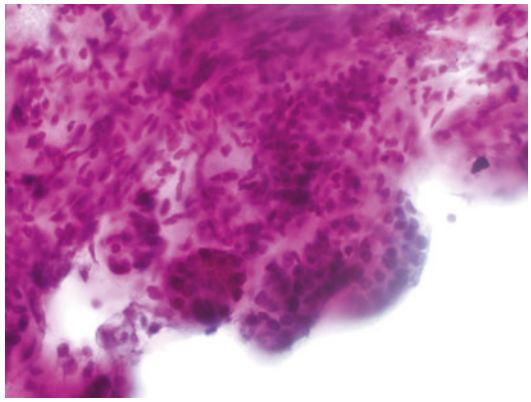
Mediastinal Tumor, Fine Needle Aspiration, Fig. 4 High power Diff-Quik stain demonstrating the pleomorphic elongated cells with moderate amount of cytoplasm, many having fibrillar extensions. The nuclei are central or eccentric and contain conspicuous nucleoli (Diff-Quik stain)



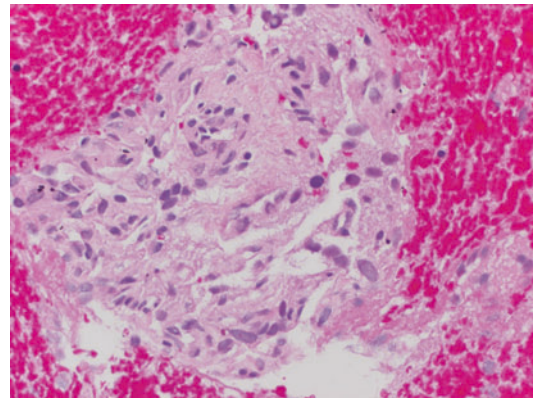
Mediastinal Tumor, Fine Needle Aspiration, Fig. 6 Low power H&E stain demonstrating the correlating biopsy of malignant peripheral nerve sheath tumor (H&E stain)

Paragangliomas generally arise in the anterior or posterior mediastinum. The cytologic findings are the same as those for adrenal pheochromocytomas (described elsewhere) (Figs. 7–9).

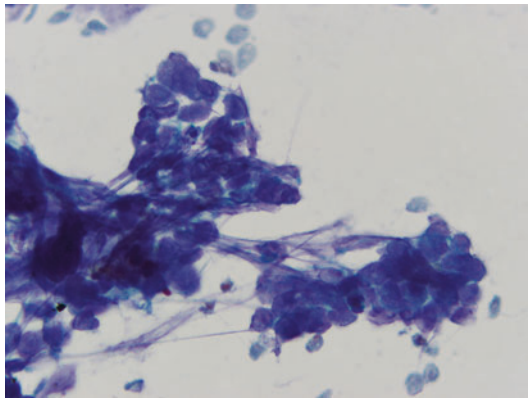
A number of mesenchymal tumors may be seen in the mediastinum. *Thymolipoma* is a rare tumor, generally affecting adolescents and young adults. The smears will show lymphocytes, epithelial cells, and mature adipose tissue. Rarely, Hassall's corpuscles may be seen. Cases of malignant transformation (liposarcoma and low-grade stromal sarcoma) have been reported. *Solitary Fibrous Tumor* (SFT) is the most common mesenchymal tumor of the mediastinum. The cytologic features of SFTs are quite diverse. The smears are variably cellular, with spindle cells seen singly and in loose clusters. The spindle cells are usually bland with uniform nuclei with inconspicuous nucleoli. A bloody background is common. Immunohistochemical staining with CD34 should be positive in the spindle cells. *Liposarcoma* is the most common malignant



Mediastinal Tumor, Fine Needle Aspiration, Fig. 7 Low power Papanicolaou stain demonstrating an FNA of malignant paraganglioma. The cellular component appears as syncytial groups of round cells admixed with many spindled cells recapitulating the "Zellballen" pattern (Pap stain)



Mediastinal Tumor, Fine Needle Aspiration, Fig. 9 Medium power H&E stain demonstrating the correlating cell-block showing a fragment of neoplastic cells with abundant granular cytoplasm and variable polygonal to elongated cells embedded in a highly vascular stroma (H&E stain)



Mediastinal Tumor, Fine Needle Aspiration, Fig. 8 High power Papanicolaou stain demonstrating small group of the neoplastic cells presenting mainly as poorly differentiated small blue cells raising a wide range of differential diagnoses (Pap stain)

mesenchymal neoplasm of the mediastinum. The smears are usually cellular with numerous variably sized adipocytes and sometimes difficult-to-find lipoblasts – malignant cells with vacuolated cytoplasm and scalloped nuclei. The lipoblast nuclei are often peripherally located, hyperchromatic, and contain prominent nucleoli. Immunohistochemistry and/or fluorescence in situ hybridization for MDM2 and CDK4 can be helpful in establishing a diagnosis of liposarcoma (Binh et al. 2005).

Other mediastinal tumors with cytologic findings described elsewhere include Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Germ Cell Tumors, Neuroblastoma, Ganglioneuroma, and PNET.

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Medullary Breast Carcinoma, Cytological Findings

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Synonyms

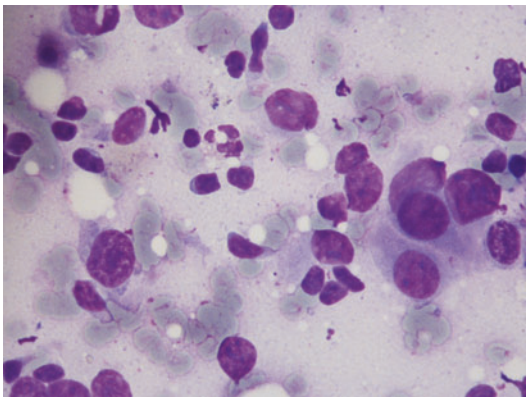
Carcinomas with medullary features

Definition

Medullary carcinomas are included in a group of tumors called "carcinomas with medullary features," characterized by the following features: circumscribed or pushing borders, a syncytial growth pattern, cells with high nuclear grade, and presence of prominent lymphoid infiltration (Fig. 1).

Clinical Features

- **Incidence**
  Variable depending of the stringency of the diagnostic criteria (1–5%).
- **Age**
  Patients tend to be relatively young with a median age around 46–54 y/o.
- **Sex**
  More frequent among women in Japan than in the USA and more frequent in black women than in white ones.



Medullary Breast Carcinoma, Cytological Findings, Fig. 1 Medullary breast carcinoma. Smear showing large cells with nuclei pleomorphism. Note lymphocytes at background (Giemsa stain)

- **Site**
  There is not any difference from other histotypes.
- **Treatment/Outcome**
  Most of these tumors are triple negative and are managed with surgery, radiotherapy, and chemotherapy.

Macroscopy

Macroscopically, this lesion is well circumscribed with a soft and uniform consistence which can be misdiagnosed as a fibroadenoma or colloid and papillary carcinoma.

Microscopy

Cytological, the cells are organized in clusters and syncytial groups with few isolated cells. The cells are medium to large and irregular in size with an increased N/C ratio with macronucleoli. The cells show basophilic and granular cytoplasms, usually scant. The cells are mixed with lymphocytes and plasma cells. The key features of these cancers are summarized as follows: (1) cellular smears with syncytial aggregates and single cells, (2) large cells with nuclei pleomorphism, and (3) benign lymphoid cell and plasma cells.

The histological pattern reveals three main morphological criteria necessary for a correct diagnosis: (1) the evidence of epithelial cells organized in syncytial sheets with large and pleomorphic nuclei and high mitotic count, represented at least around 75% of the lesion; (2) the stroma presenting lymphocytes and plasma cells; and (3) the typical pushing borders rather than infiltrative.

Immunophenotype

These tumors are often negative for estrogen and progesterone receptors and HER2 ("triple negative") and variably express cytokeratins 5/6 and 14, EGFR, P-cadherin, and P53.

Molecular Features

Most of these tumors are recognized as basal like and show genomic instability. There is a strong association between BRCA1 mutation and carcinomas with medullary features. The most frequent somatic mutation is on P53.

Differential Diagnosis

A definitive diagnosis of carcinoma with medullary features requires evaluation of tissue sections to determine margin circumscription and other parameters, so such a diagnosis on FNAC can only be suggested when the clinical and imaging findings – well circumscribed, mobile mass – coupled with the cytological study results are suggestive of this carcinoma. The cytological differential diagnoses include poorly differentiated ductal carcinoma with lymphocytic infiltrate, malignant lymphoma, and metastatic carcinoma in an intramammary lymph node. The latter represents the most difficult differential diagnosis and the clinical history may be helpful.

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Medullary Carcinoma of Thyroid, Cytological Findings

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Synonyms

C-cell carcinoma; Neuroendocrine carcinoma; Solid carcinoma with amyloid stroma

Definition

Medullary carcinoma of the thyroid is a carcinoma arising from the thyroid C-cell. Two forms have been described: a familial heritable form which represents about 25% of the medullary carcinoma, which is associated with other endocrine tumors (multiple endocrine neoplasia /MEN 2A and 2B) and a sporadic form. Whatever the form, this tumor is characterized by secretion of calcitonin.

Clinical Features

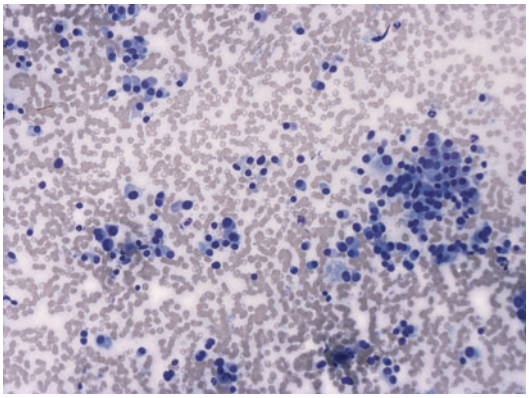
- **Incidence**
  The incidence rate is about 2–4%. These tumors represent 5% of all thyroid malignant tumors.
- **Age**
  This cancer occurs at a mean age of 50 for the sporadic form and in childhood or late adolescence/early adulthood for the familial form.
- **Sex**
  It is slightly more frequent in women than men.
- **Site**
  Medullary carcinoma may be located everywhere in the gland but more frequently in the middle-lower part of a lobe. Heritable medullary carcinomas are multifocal.
- **Treatment**
  Treatment is total thyroidectomy, usually associated with a central and bilateral neck nodes dissection. External radiotherapy or chemotherapy is reserved for recurrent tumors or very extensive ones. Radioiodine therapy is not relevant. Targeted therapies are expected.
- **Outcome**
  Prognosis is quite good for carcinomas located in the thyroid gland. The systematic thyroid gland ultrasonography in cases of MEN syndrome or personal medical history of parathyroid adenoma or pheochromocytoma allows to discover very small carcinoma and thus contributes to enhance the prognosis.

Macroscopy

The medullary carcinomas vary in size and color. The tumors may be small, 1 cm wide or less, or larger to about several centimeters wide. The color is usually gray-white but may be brown or tan. The tumor is often unique in the sporadic form and multiple/bilateral in familial form.

Microscopy

Medullary carcinoma does not differ cytologically, regardless of the familial or sporadic form.

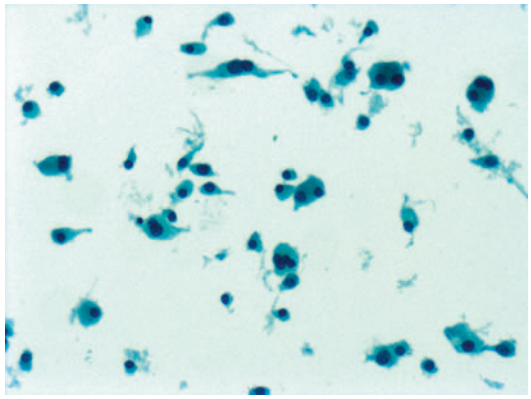


Medullary Carcinoma of Thyroid, Cytological Findings, Fig. 1 Numerous single cells with loose clusters; numerous binucleated cells; notice the plasmacytoid features (conventional; MGG staining ×25)

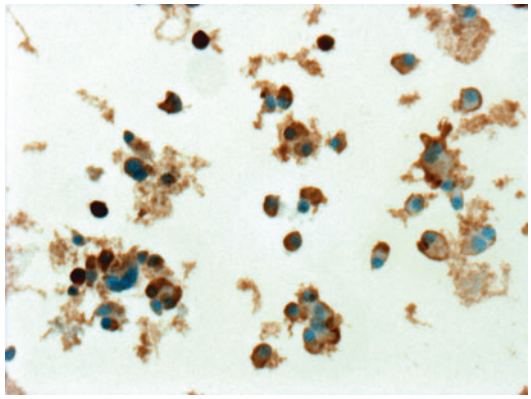
Usually, there is a high cellularity on the slides and most of the cells are isolated ones; some groups of cells may be observed but the numerous single cells are characteristic of this carcinoma. The cytoplasm is more or less abundant, basophilic, with variable shapes: Cylindric, spindle-shaped, and polygonal cells may be observed. Sometimes some pink granulations may be seen in the cytoplasm when MGG staining. The nuclei are slightly enlarged, eccentric, typically with plasmacytoid chromatin. The cells may be bi- or multinucleated (Figs. 1 and 2). Sometimes, some amounts of an amorphic material, pink with Giemsa staining too, indicate the presence of amyloid deposits. Usually, in medullary carcinoma, there are no marked atypia and anisokaryosis. Nevertheless, in poorly differentiated medullary carcinoma, cells may be atypical.

Immunophenotype

The tumoral cells are strongly positive for calcitonin (Fig. 3) and carcino-embryogenic Antigen. They are also positive for several endocrine markers such as chromogranin A, synaptophysin, etc. as well as for TTF1.



Medullary Carcinoma of Thyroid, Cytological Findings, Fig. 2 Single cells either spindle-shaped or polygonal; numerous bi-/multinucleated cells (LBC; Hologic®; Papanicolaou staining ×40)



Medullary Carcinoma of Thyroid, Cytological Findings, Fig. 3 Same case as on Fig. 2; strong cytoplasmic positivity for calcitonin (LBC; Hologic®; immunocytochemistry; Ventana. Antibody: Dako A567)

Molecular Features

Germ-line mutations in the RET proto-oncogene are characteristic for the familial medullary carcinoma. Inversely, the genetic markers of the sporadic form remain still poorly known: Several RET variants mutations have been observed in the sporadic form, leading to recommendations for a "genetic screening" in patients with medullary carcinoma in order to identify the markers in this sporadic form and to detect the rate of heritable disease among apparently sporadic cases. Finally, RAS mutations have also been found in sporadic medullary carcinoma.

Differential Diagnosis

Medullary carcinoma are usually diagnosed through its morphology and confirmed by immunocytochemistry. There is a risk of mistake essentially with the Hürthle cell tumors since the cells share some similar features such as predominant single cells arrangement, eccentric nuclei, and bi-/multinucleated cells.

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Medulloblastoma, Cytological Findings

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Synonyms

Primitive neuroectodermal tumor (PNET)

Definition

Medulloblastoma is a malignant invasive embryonal small-cell tumor.

Clinical Features

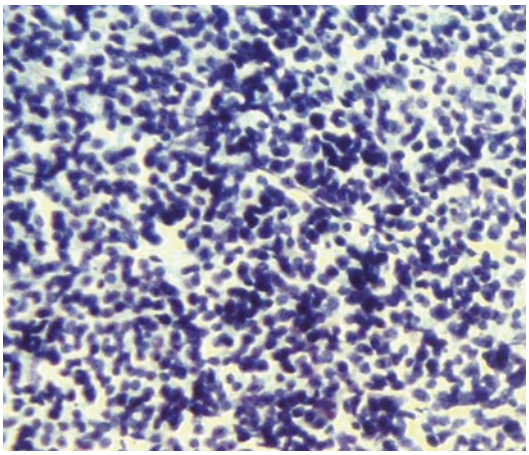
- **Incidence**
  The incidence has been estimated 0.5 per 100,000 children less than 15 years.
- **Age**
  The peak of incidence of medulloblastoma is 7 years, and more than 50% of the cases occur in younger people than 16 years.
- **Sex**
  There is a predominance of male.
- **Site**
  Medulloblastoma predominates in the cerebellum especially in the vermis and into the fourth ventricle.
- **Treatment**
  The treatment of patients with medulloblastoma involves surgery, radiotherapy, and chemotherapy.
- **Outcome**
  The survival of medulloblastoma is around 60–70% in 5 years.

Macroscopy

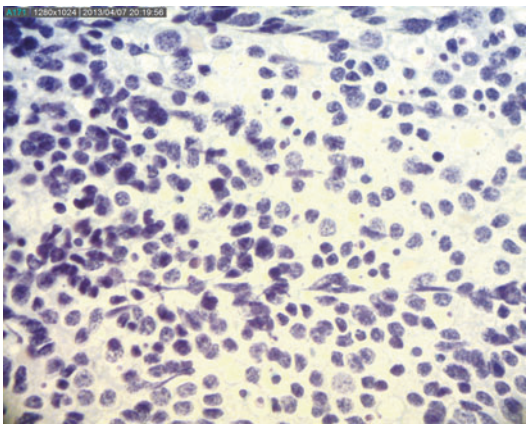
Medulloblastomas are formed by pink or gray masses well vascularized with occasional foci of necrosis and tendency to infiltrate the space subarachnoid.

Microscopy

Cytology demonstrates richly cell sheets composed of monotonous and relatively uniform densely packed cells (Fig. 1). It shows that cells have inapparent cytoplasm, hyperchromatic nuclei, occasional mitoses, and individual cell necrosis; are round, oval, carrot shaped with thick nuclear membrane (Fig. 2); and occasionally exhibit nuclear molding. The cells are distributed in cords or outline of rosettes (Fig. 3).



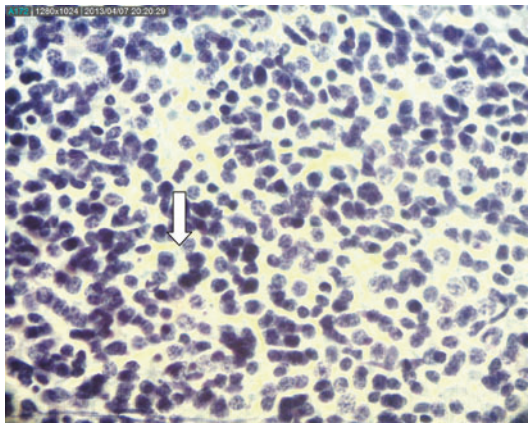
Medulloblastoma, Cytological Findings, Fig. 1 Medulloblastoma – note richly cell sheets composed of monotonous cells uniform densely packed (*toluidine blue*) (Note: Source of the figures of the authors)



Medulloblastoma, Cytological Findings, Fig. 2 Medulloblastoma – note cells with inapparent cytoplasm, hyperchromatic nuclei, and round or oval and thick nuclear membrane (*toluidine blue*) (Note: Source of the figures of the authors)

Immunophenotype

The immunophenotype of medulloblastoma includes reactivities for vimentin, nestin, and nerve growth factors.



Medulloblastoma, Cytological Findings, Fig. 3 Medulloblastoma – note cells forming rosette (*arrow*) (*toluidine blue*) (Note: Source of the figures of the authors)

Molecular Features

The main molecular alterations in medulloblastoma are isochromosome 17q, loss of chromosome 6, and trisomy of chromosome 7.

Differential Diagnosis

Potential confusion with medulloblastoma is the presence of granular layer neurons of the cerebellum normally. These are small with very scanty cytoplasm with uniform nuclei and without mitoses. Generally, there are other neurons interposed as Purkinje cells. Another differential diagnosis is with other forms of PNET (ependymoblastoma and pineoblastoma), but this depends on clinical and radiology because it is not possible to distinguish in cytological aspects.

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Melanoma, Cytological Findings

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Synonyms

Skin cancer

Definition

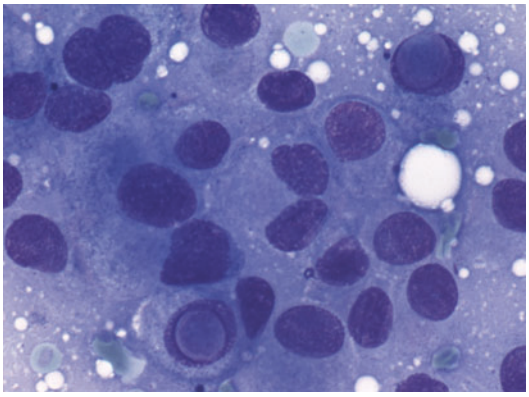
Malignant tumors originating from melanocytic cells.

Clinical Features

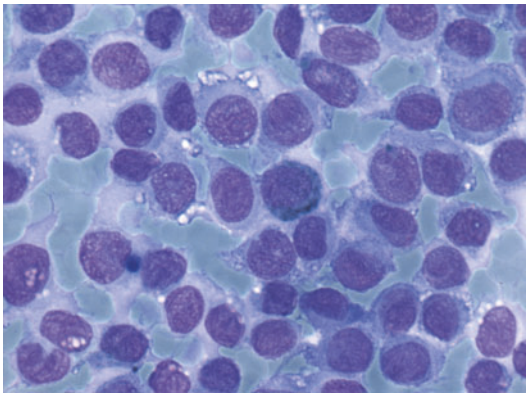
- **Incidence**
  Metastases are seen in around 20% of patients operated on for melanoma.
- **Age**
  All ages but small children.
- **Sex**
  Slight male predominance.
  Lymph nodes, liver, lung, cutis-subcutis.
- **Treatment**
  Surgery, chemotherapy, radiation.
- **Outcome**
  Metastatic melanomas are often fatal, but long-term survival occurs.

Microscopy

At least five cell types exist: epithelioid, lymphoma like, spindle cell, pleomorphic, and



Melanoma, Cytological Findings, Fig. 1 Melanoma epithelioid type: monomorphic cells with slightly irregular nuclei, two with cytoplasmic inclusion and distinct cytoplasm. The cells have a marked cohesive tendency. MGG



Melanoma, Cytological Findings, Fig. 2 Melanoma lymphoma like: monomorphic tumor cells with round nuclei and distinct often vacuolated cytoplasm, few cells with pigment. There is little cohesive tendency. MGG

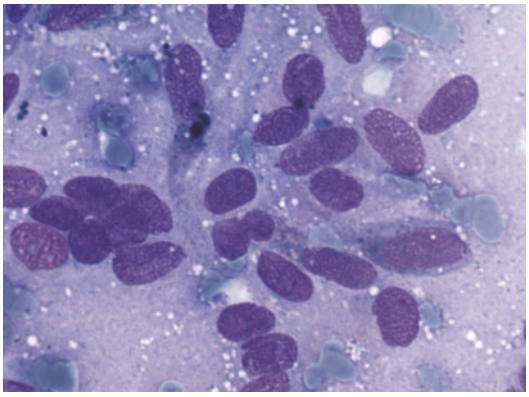
clear cell type (Figs. 1–5). The cytoplasm is usually rich and often contains melanin but can also be finely vacuolated.

Immunophenotype

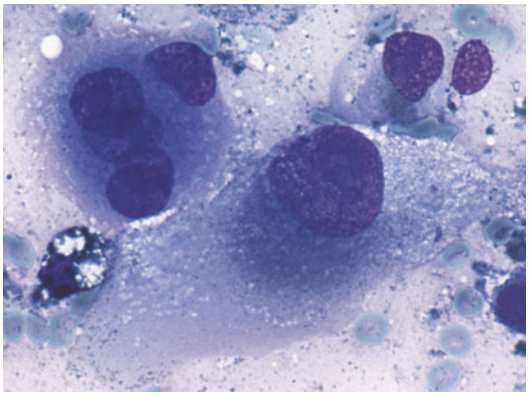
Vimentin, HMB45, S-100, and melan A positive.

Molecular Features

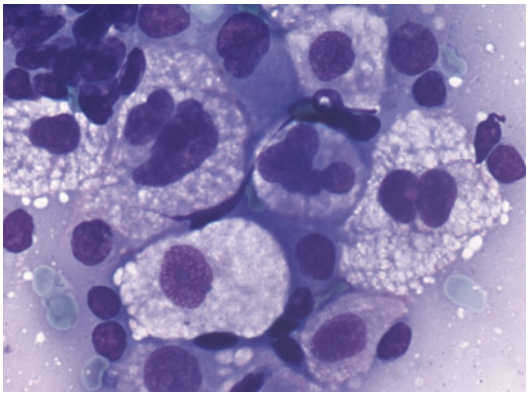
No consistent findings.



Melanoma, Cytological Findings, Fig. 3 Melanoma spindle cell type: cells with oval to spindle nuclei and sparse cytoplasm with pigment. MGG



Melanoma, Cytological Findings, Fig. 4 Melanoma pleomorphic type: pleomorphic tumor cells with macronuclei or multiple nuclei and rich cytoplasm with pigment. MGG



Melanoma, Cytological Findings, Fig. 5 Melanoma clear cell type: tumor cells with pleomorphic nuclei and cytoplasm with numerous vacuoles. MGG

Differential Diagnosis

Metastasis from most malignant tumors and lymphoma.

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Meningioma of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

Benign tumor of meningoendothelial cells.

Clinical Features

- **Incidence**
  Uncommon.
- **Age**
  Middle age, rarely observed in children.
- **Sex**
  Incidence rate slightly higher for females than for males.
- **Site**
  Orbit.
- **Treatment**
  Surgery.
- **Outcome**
  Favorable.

Macroscopy

► Meningioma of the orbit may arise from the meningoendothelial cells which envelop the intraorbital tract of the optic nerve or present as an orbital extension of intracranial meningioma.

Microscopy

Smears show typical whorls of polygonal or spindle-shaped meningoendothelial cells with large cytoplasm without the "empty oval" vacuolated nuclei which may be observed in histological sections; psammoma bodies may also be seen.

Cross-References

► Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
► Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
► Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
► Conjunctiva Cytology, General Aspects
► Conjunctival Inflammatory Lesions, Cytological Findings
► Conjunctival Lymphoma, Cytological Findings

- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Gland Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Meningioma, Cytological Findings

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Synonyms

Benign meningioma; Meningiomatosis

Definition

Meningothelial cell neoplasms attached to the inner surface of the dura mater essentially benign, have a low rate of proliferation and well-circumscribed mass.

Clinical Features

- **Incidence**
  The occurrence of meningiomas ranges from 10% to 30% of intracranial tumors.

- **Age**
  Meningioma occurs most commonly in middle-aged and elderlyl with peak during sixth and seventh decades.
- **Sex**
  The lesions predominate in females with rations of 3.5:1 in the middle age.
- **Site**
  Most lesions were located in the following regions: intracranial, intraspinal, or orbit.
- **Treatment**
  The treatment of choice is surgery with excision entire lesion. Occasional recurrences may occur when the lesion is not completely excised.
- **Outcome**
  The biggest problem related to meningiomas is the recurrence. The most important factors related to recurrence are clinical factors and involve the tumor location, extent, and invasion of structures and complete excision of the lesion.

Macroscopy

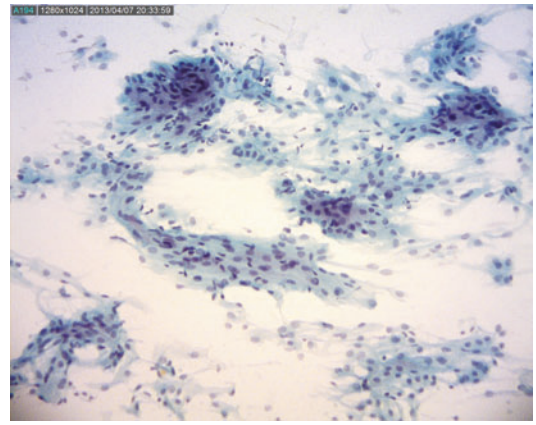
Most meningiomas are rounded masses, well-demarcated, reddish, and firmly linked to dural meninges. Invasion of underlying dura or of dural sinuses is quite common.

Microscopy

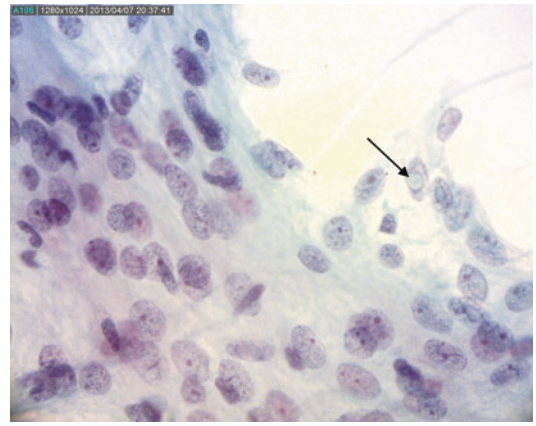
Meningioma presents uniform cells and with imprecise limits (Fig. 1), with round nuclei and delicate, evenly distributed chromatin with one or more small nucleoli and some intranuclear cytoplasmic inclusions (Fig. 2). Concentric whorl formations, sometime calcified, and syncytial appearance of the tumors cells were also seen.

Immunophenotype

The meningiomas usually show high positivity for epithelial membrane antigen (EMA) and vimentin and variable positivity for S-100 protein.



Meningioma, Cytological Findings, Fig. 1 Meningioma – Note clusters with irregular margins and uniform cells with imprecise limits (Papanicolaou)



Meningioma, Cytological Findings, Fig. 2 Meningioma – Note round nuclei and delicate, evenly distributed chromatin with one or more small nucleoli and intranuclear inclusions (*arrow*) (Papanicolaou)

Molecular Features

The most consistent genetic alteration of meningiomas is related to the deletion of chromosome 22. Until 60n% of sporadic meningiomas may have a mutation in the NF2 gene.

Differential Diagnosis

The main differential diagnoses are metastatic carcinoma, Schwannoma, and hemangiopericytoma.

Carcinomas generally have coarse chromatin and nuclear atypia which contrasts with the benign aspects of meningioma. The Schwannomas due to aspect fascicular of the cells and benign pattern is of difficult distinction as meningioma. Will be necessary good correlation of clinical and lesion topography. Hemangiopericytoma is distinguished by no feature related to meningiomas as cells in the lobules, or whorls and psammoma bodies. They present smears with highly cellular thick and irregular clusters of cells with relatively uniform round or oval, plump nuclei.

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Mesothelial Cells, Reactive

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Synonyms

Benign mesothelium

Clinical Features

Not applicable

Definition

Mesothelial cells are mesodermally derived epithelial cells that line body cavities (pleura, pericardium, and peritoneum). Under normal conditions, mesothelial cells form a flat, single, uniform layer. When these surfaces become irritated or injured, mesothelial cells can proliferate and take on a variety of morphologic and cytologic appearances.

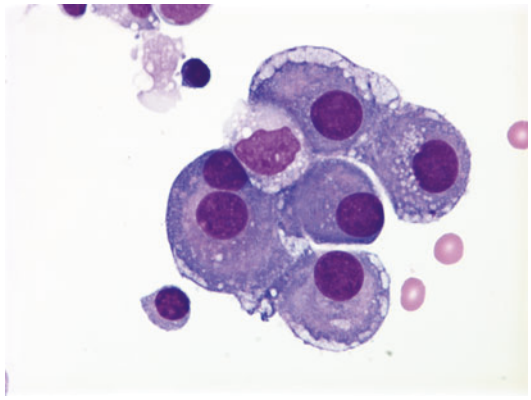
There are many causes of reactive mesothelial cells including: infection, inflammation, infarction, liver disease, radiation, chemotherapy, systemic disease, trauma, foreign materials, and neoplasia (DeMay 2012). Certain conditions like pulmonary embolism and infarction, active liver disease, uremia, pancreatitis, acute inflammation, long-term dialysis, and radiation/chemotherapy are known to cause markedly reactive mesothelial cells that resemble malignancy (DeMay 2012).

Macroscopy

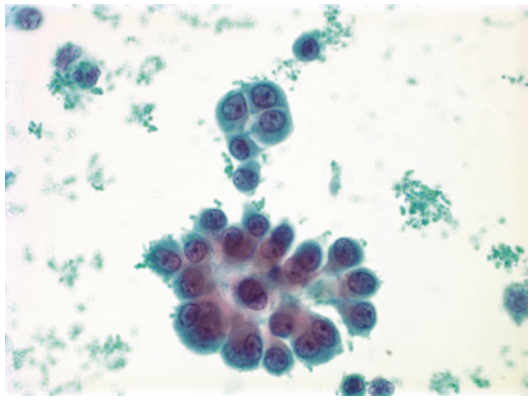
Fluids with reactive mesothelium may vary grossly from thin and clear fluids to thick and turbid fluid, depending on the underlying etiology and other cellular components.

Microscopy

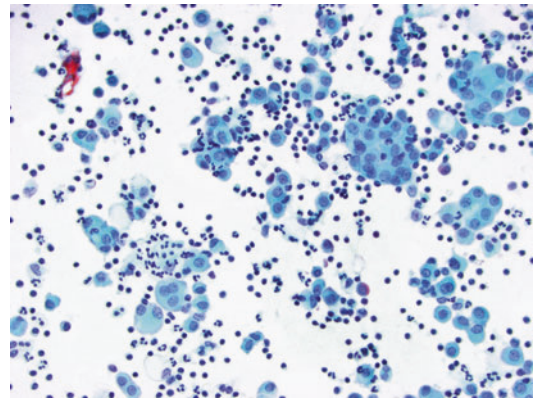
Mesothelial cells may show a wide size variation, but average about 20 μm in diameter and range from 15 to 30 μm. They may present as single isolated cells or form small cell groups (Fig. 1). Sheets of orderly arranged mesothelial cells are commonly seen in peritoneal washings, but this is an uncommon finding in effusions. When mesothelial cells come together, slit-like spaces are often seen between two adjacent mesothelial cells (Fig. 2). These so-called "windows" offer a clue to their mesothelial origin; however, it should also be noted that "window-like" space can be seen between adjacent cells of adenocarcinoma. In this case, the slit-like space is usually composed of secreted mucin. Mesothelial cell clusters often have a knobby,



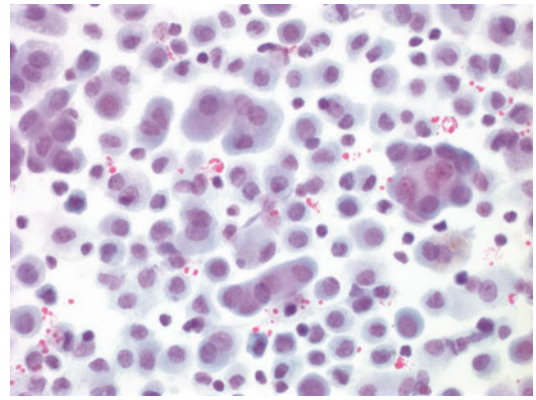
Mesothelial Cells, Reactive, Fig. 1 High-power Romanowsky stain demonstrates loosely aggregated benign mesothelial cells with intercellular windows. Notice the abundant two-tone cytoplasm, submembranous glycogen vacuoles, and the benign centrally located nuclei with inconspicuous nucleoli. Occasionally, binucleated cell is present (Romanowsky stain)



Mesothelial Cells, Reactive, Fig. 2 High-power Papanicolaou stain demonstrates clusters in a reactive pleural effusion. Notice the knobby contours of the clusters. The nuclei are slightly enlarged and contain more prominent nucleoli. Several cells are multinucleated (Pap stain)



Mesothelial Cells, Reactive, Fig. 3 Medium-power Papanicolaou stain demonstrates a reactive parapneumonic effusion. There is an overall monotonous mesothelial population present as single cells and clusters in a background of neutrophils (Pap stain)



Mesothelial Cells, Reactive, Fig. 4 Medium-power Papanicolaou stain of a reactive effusion in a patient with cirrhosis. Notice the monotonous population of mesothelium forming clusters (Pap stain)

lobulated, or flower-like border while clusters of adenocarcinoma typically have smooth community borders. However, this rule is not universal as clusters of adenocarcinoma can occasionally have a knobby contour. When mesothelial cells are injured or irritated, they may proliferate and form spherical groups, sometimes with papillary architecture, with hyperchromasia, multinucleation, distinct nucleoli, frequent mitotic figures, and cytoplasmic vacuoles (Figs. 3 and 4). These cells may be indistinguishable from cells of adenocarcinoma. In these cases, immunocytochemistry can be of great help in distinguishing atypical reactive mesothelial cells from adenocarcinoma.

Mesothelial cells tend to have a constant nuclear-to-cytoplasmic (N/C) ratio, but can range in size from small cells with high N/C ratios to large cells with low N/C ratios.

Mesothelial cells usually have one nucleus, ranging in size from 8 to 14 μm, but not uncommonly can be binucleated, or multinucleated especially in reactive conditions. The nucleus is oval

in shape and centrally located. Occasionally, the nucleus may be eccentrically located. The nuclear membrane is typically prominent and smooth. The chromatin pattern is normally finely granular and even. In reactive conditions, the chromatin may become coarse; however, it should still remain uniform in distribution. Nucleoli range from small and indistinct to prominent in reactive conditions, but multiple or macronucleoli are findings suggestive of malignancy. Normal appearing mitotic figures may be encountered, while atypical mitotic figures are more suggestive of malignancy.

The cytoplasm of mesothelial cells has a characteristic staining pattern. In Papanicolaou-stained smears, the perinuclear cytoplasm is dense due to accumulation of intermediate filaments, while the periphery of the cytoplasm is lacy due to the presence of microvilli. In Romanowsky-stained preparations, the perinuclear zone stains paler while the outer zone is denser. The mesothelial cell cytoplasm is commonly vacuolated. These vacuoles are typically degenerative in nature and consist of water and electrolytes (DeMay 2012). Other types of cytoplasmic vacuoles consist of lipid that occur near the nucleus, and submembranous or peripherally located vacuoles that contain glycogen. Cells that have secretory vacuoles containing epithelial mucin are indicative of malignancy.

Differential Diagnosis

As previously described, reactive mesothelial cells can mimic adenocarcinoma or mesothelioma. Despite the challenges, there are cytomorphologic features that are helpful in distinguishing benign from malignant effusions, and in difficult cases, immunocytochemistry is of value.

When evaluating fluids, the first step is to assess the cellularity and complexity of the groupings at low power. Specimens with high cellularity with many large clusters of cells favor a diagnosis of malignancy while benign effusions show smaller, looser collections of cells with less complex groupings. Identifying clusters of cells with well-formed glandular formations or papillae is also more likely to be malignant. Next, one should look at the cytologic features. Usually, in effusions harboring metastatic carcinoma, one is able to appreciate a distinct two cell population. One population consists of the native mesothelial cells, and the other group consists of a discrete foreign population of cells. Mesothelial cells, even in their most reactive forms, usually bear some resemblance to one another. If the atypical cells are attached to clearly benign mesothelial cells, it is favored that the cells in question are of mesothelial origin. Mesothelial cells typically form knobby, flower-like clusters, with cell-in-cell arrangements and windows. In contrast, cells of adenocarcinoma form tighter groups with shared borders and eccentric, hyperchromatic nuclei with coarse chromatin and delicate cytoplasm. In difficult cases, immunocytochemistry is of value.

The distinction between reactive mesothelial cells and mesothelioma is a problematic area in effusion cytology. Generally, larger cells within larger cell clusters favor a diagnosis of mesothelioma over reactive mesothelial cells. While some cases of malignant mesothelioma may show overt nuclear atypia, many of them will not have clear-cut malignant features. Thus, the overall cellularity and architectural features are more important than nuclear atypia in distinguishing reactive mesothelial cells from *mesothelioma* (Pereira et al. 2006).

Immunocytochemistry

A myriad of immunocytochemical markers has been used and studied to attempt to differentiate mesothelial cells from carcinoma. There is no one marker that is sufficiently sensitive and specific enough to distinguish between reactive mesothelial cells and carcinoma; therefore, the approach that most cytopathologists have taken is to use a panel of stains to confirm their cytomorphologic impression. This panel usually consists of two mesothelial markers and two carcinoma markers.

The two most common mesothelial cell markers are calretinin and D2-40. Other

markers like WT-1 and CK5/6 can also highlight mesothelial cells, but may show some overlap with specific carcinomas. Traditional markers used to highlight the presence of carcinoma are MOC-31, Ber-EP4, CEA, B72.3, and Leu-M1 (CD15). Special stain mucicarmine can also be performed and is negative in cells of mesothelial origin and positive in adenocarcinoma.

Immunocytochemistry is of limited value in distinguishing reactive mesothelial cells from mesothelioma. p53, EMA (thick membranous staining), E-cadherin, and Glut-1 have been shown to be positive in mesothelioma and negative in benign mesothelial proliferations while desmin has been shown to be positive in benign and negative in malignant mesothelial proliferations (Hasteh et al. 2010).

Molecular Testing

Fluorescence in situ hybridization for homozygous loss of p16 (CDKN2A) in cytology preparations has been shown to occur in some mesotheliomas. There are a number of technical limitations however, and most importantly, only a proportion of mesotheliomas show a homozygous loss of p16 (Churg and Salle-Galateau 2012). Thus, molecular assays are of somewhat limited value at this time in the diagnosis of mesothelioma.

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Mesothelioma, Effusions

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Synonyms

Malignant mesothelioma

Definition

Primary malignant effusion arising in any serosal cavity and originating from mesothelial cells. Mesothelioma is one of very few malignancies that have direct association with asbestos exposure which is documented in over 80% of reported cases.

Clinical Features

Most patients initially present with shortness of breath as a result of the large pleural effusion. When the mesothelioma invades the chest wall or diaphragm, the patient will experience chest pain as well. Other symptoms include fatigue, dry cough, fever, and weight loss.

The presenting symptoms tend to be vague and insidious, and consequently, it is not uncommon for the diagnosis to be established 3–6 months later.

- **Incidence**
  The incidence of mesothelioma is reported as 2,500 cases in USA and 5,000 cases in Western

Europe annually. It is projected that mesothelioma incidence will reach a peak by 2020 worldwide.

- **Age**
  Mesothelioma has a long latency period after the asbestos exposure that may reach up to four decades. It commonly manifests around age 60 although patients with childhood exposure to asbestos may present earlier.
- **Sex**
  Mesothelioma is a male-predominant disease.
- **Site**
  Mesothelioma can occur in any body cavity lined by a serosal surface epithelium. The site of occurrence in descending order is pleural, peritoneum, pericardium, and tunica vaginalis testis. The ratio of the incidence in pleura versus peritoneum in the literature ranged from 3:1 to 11:1. It is important to recognize that pleural effusions tend to be unilateral in about 95% of cases and bilateral in only 5% of cases. The right pleura is affected in about 60% of patients.
- **Treatment**
  Extrapleural pneumonectomy may be considered in patients with epithelioid mesothelioma and documented negative mediastinal lymph nodes based on biopsy and mediastinoscopy. Unfortunately, this method of treatment has been beneficial in only 10–15% of patients. Chemotherapy with the combination of cisplatin and premetrexed has been reported to offer better survival advantage (12 months) and is currently preferred as first line of treatment for those not eligible for surgery. Radiation therapy is only used to control local chest wall invasion.

  Because peritoneal mesothelioma has a localized nature, loco-regional therapies have been explored. The initial therapy is usually cytoreduction to remove all grossly visible tumor. This is followed by intraoperative, intraperitoneal hyperthermic perfusion of high-dose chemotherapy uniformly to the entire peritoneal surface. Hyperthermia is believed to enhance the cytotoxic effect of multiple chemotherapeutic agents.
- **Outcome**
  Untreated, mesothelioma has very bad prognosis with only 6-month survival. Survival rate of up to 5 years has been reported with recent treatment regimens including surgery and chemotherapy.

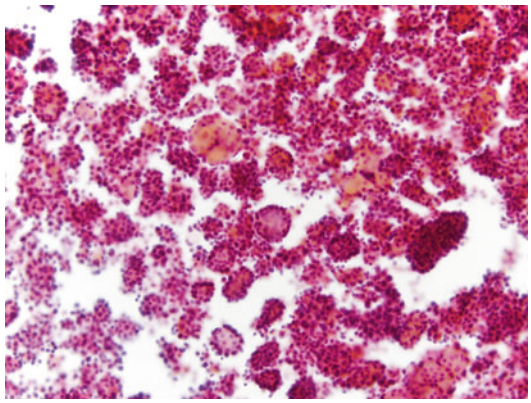
Macroscopy

Effusions of malignant mesothelioma tend to be turbid and thick in consistency, frequently described as "Tar-like" or "Honey-like" consistency (Michael 2012).

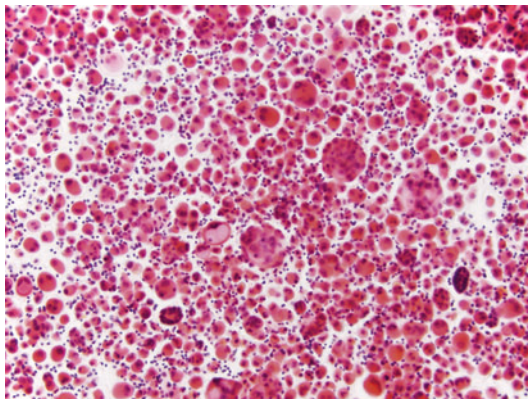
Microscopy

A large effusion is present in up to 95% of cases of mesothelioma, most commonly a pleural effusion. Later in the disease, the parietal and visceral pleura fuse and the effusion disappears. Literature suggests that mesothelioma can be diagnosed in effusion specimens in up to 50% of cases by cytologic features alone, and up to 80% with immunohistochemical staining (Ismail-Khan et al. 2006). Not all malignant mesothelioma effusions contain cells, however; 10% may be bloody and acellular.

The cytologic features of malignant mesothelioma cells overlap with *reactive mesothelial cells*, although they tend to be much more prominent in mesothelioma. De May describes the clusters in mesothelioma as "more and bigger cells in more and bigger clusters" (De May 2012). Cell windows are seen between cells in cords and small clusters. Cellular clasping, pinching, and cell within cell arrangement are pronounced. Clusters and spheres of cells with scalloped borders also referred to as morules or berry-like clusters are present (Pereira et al. 2006). Cells will show two-toned cytoplasm with a brush-like border and submembranous glycogen vacuoles. The nucleus is centrally located and multinucleation is a common finding. Features more typical of epithelioid mesothelioma, the most common variant, include a single cell population with a wide variation in the size of the cells.



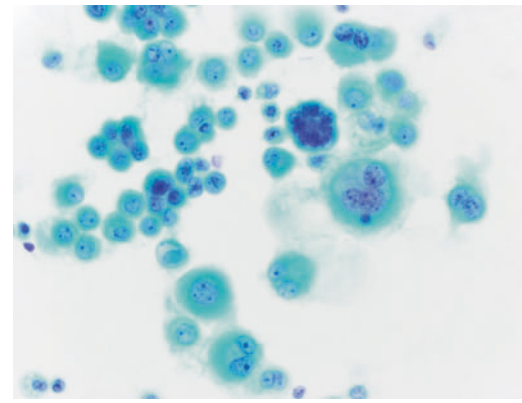
Mesothelioma, Effusions, Fig. 1 Low power Papanicolaou stain demonstrating a mesothelioma presenting as predominantly cellular clusters occasionally with collagen cores (Pap stain)



Mesothelioma, Effusions, Fig. 2 Low power Papanicolaou stain demonstrating a mesothelioma presenting with both clusters and single cells (Pap stain)

The specimen is typically high in cellularity with a bloody background. Chronic inflammation is often seen, but acute inflammation is not common. The cell pattern can be predominately cohesive groups, single cells, or a mixture of both (Figs. 1, 2, and 3). Large branching and papillary clusters may be seen. The nuclear/cytoplasmic ratio is low, and the cytologic atypia is usually mild to moderate. The chromatin pattern is slightly coarse, with irregular nuclear membranes and prominent nucleoli. Mitotic figures are seen but are not conspicuous (Michael 2012).

The sarcomatoid and biphasic variants are rare and seldom exfoliate malignant cells into fluids. Occasional malignant spindled cells or large, highly atypical cells may be seen. The small cell variant has features resembling *small cell carcinoma* including nuclear molding, but has the immunophenotype of mesothelioma. The lymphohistiocytoid variant consists of lymphocytes and histiocyte-like mesothelial cells. The deciduoid variant has highly atypical cells that are difficult to discern as mesothelial cells without immunostaining. Papillary mesothelioma is typically seen in peritoneal washes or fine needle aspirates and contains papillary groups with complex branching and collagen cores (Michael 2012).

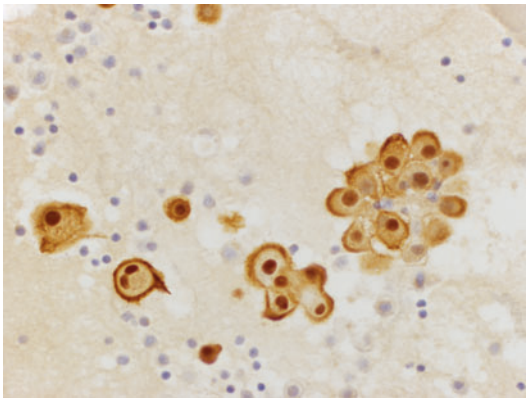


Mesothelioma, Effusions, Fig. 3 High power Papanicolaou stain demonstrating malignant mesothelioma with predominantly single cells. Notice the wide variation of size of cells and the scattered gigantic ones (Pap stain)

Immunophenotype

There is no one marker that can reliably distinguish mesothelioma from adenocarcinoma or reactive mesothelial cells, so it is important to use a panel of stains including mesothelial markers and carcinoma markers.

Mesothelin stains up to 100% of mesothelioma cases. However, mesothelin also stains a fair percentage of adenocarcinomas. *Calretinin* is one of the most sensitive stains for mesothelioma, staining up to 90% of epithelioid and 30–60% of sarcomatoid mesotheliomas (Fig. 4). The classic staining pattern is "fried egg," due to staining of



Mesothelioma, Effusions, Fig. 4 High power demonstrating calretinin stain in malignant mesothelioma with fried egg pattern (nuclear and membranous stain)

the nucleus and cytoplasm. *Wilms tumor-1 protein* (WT-1) is strongly positive in the nuclei of up to 90% of mesotheliomas; however, one must exercise caution as it is strongly expressed in ovarian and peritoneal carcinomas as well. *Podoplanin A* and *D2-40* stain the cytoplasm of 90% of epithelioid and 57% of sarcomatous mesotheliomas. *Cytokeratin 5/6* stains the cytoplasm of the majority of mesotheliomas but also most squamous cell carcinomas. *Pancytokeratin* and *cytokeratin-7* are strongly positive but of little use due to lack of specificity. *Cytokeratin-20* is variably expressed (Westfall et al. 2010).

Some of the most useful stains that are negative in mesothelioma include *carcinoembryonic antigen* (CEA), which stains most adenocarcinomas and some squamous cell carcinomas, and *B72.3*, which stains over 80% of adenocarcinomas. Other routinely negative stains in mesothelioma include *CD15* (Leu-M1), *MOC-31* (positive in up to 10%), *Ber-EP4* (positive in up to 20%), *BG-8*, and *PAX-8* (Westfall et al. 2010).

Molecular Features

Malignant mesothelioma has been shown to express distinctive major pathways and molecular signatures. It was also reported that the molecular profile in epithelioid mesothelioma reflects a more differentiated tumor when compared to the sarcomatoid variant. The most significant molecular feature currently applicable for diagnosis is the deletion of the 9p21region which is the encoding location for p16 (INK4a) by FISH analysis.

Differential Diagnosis

When the atypia of a mesothelioma in an effusion is mild, the main differential diagnosis is with *reactive mesothelial cells*. Mesothelium can be floridly reactive, with high cellularity, many clusters, and multinucleated cells. Helpful morphologic features include the wide variation in cell size of mesothelioma as compared to reactive mesothelial cells, as well as giant cells and numerous multinucleated cells that are characteristic of mesothelioma. The cells clusters are innumerable in mesothelioma compared to reactive mesothelium, and the clusters are crowded. Immunostaining for *desmin* and *EMA* may be helpful for differentiation between mesothelioma and reactive mesothelium. *Desmin*, which is positive in benign mesothelial cells, will gradually be lost as the mesothelium becomes malignant. *EMA* is not expressed in benign mesothelium but is expressed in most mesotheliomas. Otherwise, reactive mesothelium expresses all the same immunomarkers as mesothelioma.

Distinguishing mesothelioma from *metastatic adenocarcinoma in effusions* is usually more straight-forward. Generally speaking, mesothelioma at low power should appear monotonous, whereas adenocarcinoma will show a two-cell population of carcinoma cells and benign mesothelial cells. Mesothelioma typically has a low degree of atypia compared to the pleomorphism and definitive malignant characteristics of most adenocarcinomas (Pereira et al. 2006). Certain adenocarcinomas, however, can be difficult to distinguish from mesothelioma, and immunostains are quite helpful in differentiating. Breast carcinoma can present as cellular spheres with only a few single cells; however, the clusters tend to be very large as compared to mesothelioma. The presence of multinucleated cells and giant cells will help to identify mesothelioma.

Breast carcinoma will be positive for adenocarcinoma markers such as B72.3, as well as mammaglobin and often estrogen receptors, and negative for mesothelial markers. Lung adenocarcinoma may also present with high cellularity and numerous cell clusters; positive staining for adenocarcinoma markers such as *B72.3* and the lung-specific *TTF-1* will help differentiate. Primary adenocarcinoma of the serosal surface may be difficult to differentiate from peritoneal mesothelioma, particularly as there is some overlap in immunostaining. A panel of stains for both mesothelioma and serous carcinoma such as calretinin, Ber-EP4 or MOC-31, B72.3, and PAX-8 would be helpful in differentiating the two entities.

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Metanephric Adenoma, Cytological Findings

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Synonyms

Embryonal adenoma; Metanephroid renal tumor; Nephroblastoma-like adenoma of the kidney

Definition

Metanephric adenoma is a very rare benign renal tumor that occasionally affects children. These tumors are part of a spectrum of tumors composed by different proportions of the epithelium and mesenchymal/stromal tissue (metanephric adenoma, metanephric adenofibroma, or metanephric stromal tumor). The idea that some authors advocate that this tumor may be related with papillary renal cell neoplasms is controversial (Pins et al. 1999).

Clinical Features

- **Incidence**
  Metanephric adenoma occurs in both adults and children. It accounts for about 0.2% of adult renal epithelial neoplasms (Pins et al. 1999).
- **Age**
  It affects mostly teenaged patients but it has been described between 5 months and 36 years (Pins et al. 1999).
- **Sex**
  Male-to-female ratio is of 2:1.
- **Site**
  Kidney.
- **Treatment**
  If asymptomatic metanephric adenomas can be left in place, no treatment is needed. However, if the patient is symptomatic with pain or hematuria, the treatment of choice is excision.
- **Outcome**
  Most cases represent incidental findings. Some cases, however, may course with polycythemia, abdominal pain, or hematuria. So far, despite the large size that these tumors can assume, metanephric adenomas were considered to be benign, slowly growing, and non-metastasizing tumors with an excellent prognosis. Only recently two cases of metastasized metanephric adenomas were published.

  On ultrasound the tumor appears as a hyperechoic mass due to the presence of psammomatous calcifications.

Macroscopy

Metanephric adenoma can reach big sizes. The majority of these tumors are well circumscribed with a firm tan-brown cut surface. Some are partially cystic transformation, necrotic foci, hemorrhage, and calcification can be seen (Pins et al. 1999).

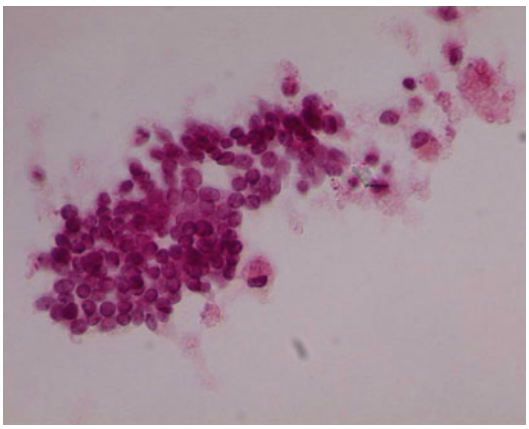
Microscopy

Metanephric adenomas are usually not encapsulated but may have a thin and discontinuous pseudocapsule. Metanephric adenoma is a highly cellular tumor that reproduces the developing metanephric tubular epithelium. It is composed of small, tightly packed, embryonal-looking tubules and acini with little intervening stroma. Occasionally, these structures coalesce forming solid sheets. In some cases small blunt papillary structures simulate immature glomeruli. Psammoma bodies are commonly seen. Metanephric adenoma does not show infiltrative growth, vascular invasion, or mitoses (Pins et al. 1999). In our opinion, the histopathologic features of MA are well reflected on cytology, allowing its recognition. Smears show a monomorphous small blue cell population in a clean background (Fig. 1) (Jiménez-Heffernan et al. 2009; Portugal and Barroca 2008). Numerous regular naked nuclei are usually present. Cells arrange themselves in clusters, tubules, glands, pseudopapillae, or morules (Jiménez-Heffernan et al. 2009; Portugal and Barroca 2008). The cells with round or oval nuclei show no atypia and a minimum amount of cytoplasm. The chromatin is dense and regularly distributed and the nucleolus is inconspicuous (Jiménez-Heffernan et al. 2009). There is no necrosis in the background and mitoses are hardly seen. Psammoma bodies can be seen in the center of tubular structures (Fig. 2) (Jiménez-Heffernan et al. 2009; Portugal and Barroca 2008).

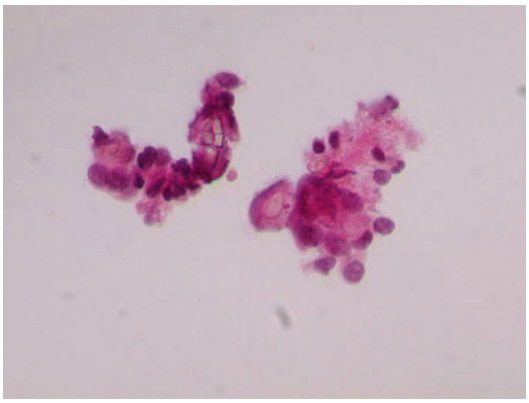
Immunophenotype

The literature suggests that MA does not have a consistent immunoprofile. Most cases are Cluster Differentiation CD56 negative. Diffuse positivity is found with CD57, Wilms tumor antibody WT1, and with URO-2 monoclonal antibody. CK7 can be focally positive and vimentin stains in the solid areas.

This immunoprofile helps in the differential diagnosis with papillary renal cell carcinoma (RCC) (Portugal and Barroca 2008).



Metanephric Adenoma, Cytological Findings, Fig. 1 In this figure a group of epithelial cells is seen. No specific morphologic characteristics are found that can help, in this picture, distinguish it from an epithelial counterpart of Wilms tumor (H&E 100×)



Metanephric Adenoma, Cytological Findings, Fig. 2 Psammoma bodies are seen in the center of tubular epithelial structures (H&E 400×)

Molecular Features

Metanephric adenoma seems to have no consistent molecular alteration and most of the times bare a normal karyotype (Brunelli et al. 2003). This knowledge confronts a previous supported hypothesis that metanephric adenoma might be related to papillary renal cell neoplasia (Brunelli et al. 2003).

Differential Diagnosis

In cytology, metanephric adenoma should be distinguished from a nephroblastoma (epithelial predominant) (Fig. 1) and from a ► papillary RCC (Jiménez-Heffernan et al. 2009; Portugal and Barroca 2008).

In the first case attention should be given to the clinical setting, patient's age, as well as to an exhaustive tumor sampling. Pure epithelial Wilms tumors are rare. Immunostains are helpfull distinguishing metanephric adenoma from wilms tumor as in this last one epithelial cells are CD56 positive and CD57 negative. WT1 antibody stains both entities and is not of a great help.

Metanephric adenoma and papillary renal cell carcinoma can share cytologic architectural features. Papillary carcinoma cells are larger and show moderate amounts of cytoplasm, sometimes vacuolated or containing hemosiderin. Macrophages are frequently present in papillary carcinoma and are rare in MA.

Differential diagnosis with solid papillary RCC can be done with immunocytochemistry and molecular profile. The presences of multicentricity, micronodular pattern, variable N/C ration, mitoses, and diffuse immunostains for CK7 and epithelial membrane antigen (EMA) as well as the presence of trisomy 7 and 17 and sex chromosome loss also favor papillary RCC (Portugal and Barroca 2008).

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Metaplastic Breast Carcinoma, Cytological Findings

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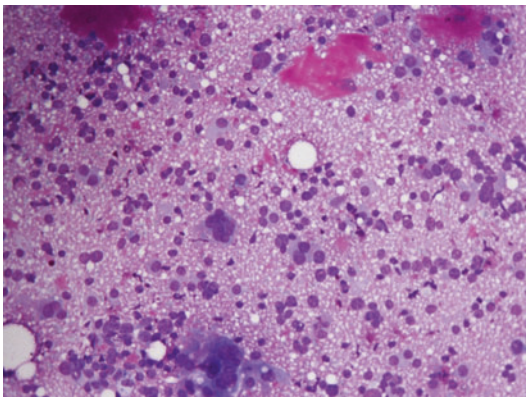
Synonyms

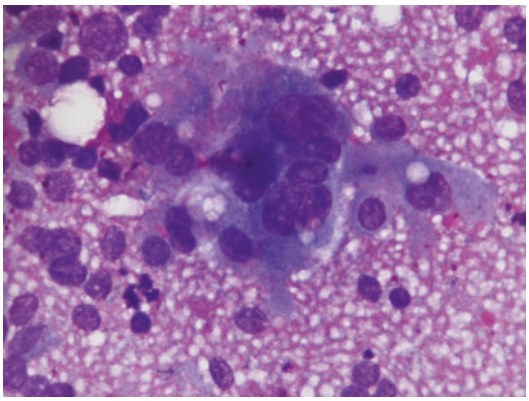
Carcinosarcoma; Sarcomatoid carcinoma; Spindle cell carcinoma; Spindle cell metaplastic carcinoma; Adenosquamous carcinoma; Squamous cell carcinoma

Definition

Metaplastic carcinoma includes a group of neoplasms characterized by differentiation of the neoplastic epithelium into squamous cells and/or mesenchymal-looking elements, in addition to epithelial components, elements such as cartilage, bone tissue, myxoid stroma, or rhabdomyoid



Metaplastic Breast Carcinoma, Cytological Findings, Fig. 1 Metaplastic breast carcinoma. High cellular smears showing atypical cells, myxoid background, and chondromyxoid stroma (Giemsa stain)



Metaplastic Breast Carcinoma, Cytological Findings, Fig. 2 Metaplastic breast carcinoma. Multinucleated malignant cell (Giemsa stain)

cells. The metaplastic spindle and squamous carcinomas may be present as either unique component or mixed with carcinoma component (Fig. 1) .

Clinical Features

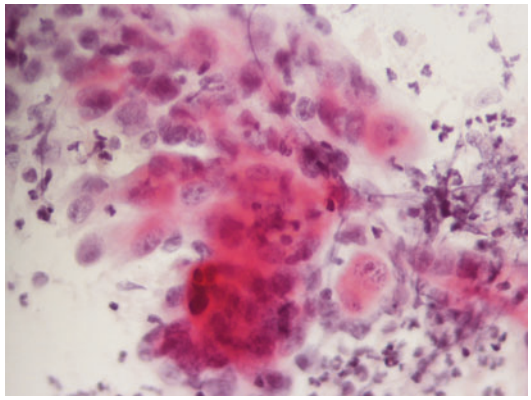
- **Incidence**
  These tumors are very rare and represent around 0.3–5% of all invasive breast cancers. If the evaluation includes only tumors with mesenchymal metaplasia, the entity accounts approximately 1% of invasive breast cancer.
- **Age**
  The average age is around 50 y/o.
- **Sex**
  The higher evidence is in female patients.
- **Site**
  There is not a specific site location.
- **Treatment/Outcome**
  It is difficult to assess the best treatment and the prognosis because of their rarity, lower responses rate to conventional adjuvant chemotherapy than those of other triple-negative tumors. Lymph-node metastases are significantly less frequent than other breast cancers even if the majority are referred as high aggressive variant with distant metastases (mainly the brain and lung) and worse prognosis.

Macroscopy

The gross appearance of metaplastic carcinomas is not distinctive and they can be well circumscribed or with irregular and indistinct borders. Cystic degenerative changes can be frequent. They tend to be relatively large tumors with a mean size of 3.9 cm ranging from 1.2 to 10 cm. They may result in nipple displacement and ulceration through the skin (Fig. 2).

Microscopy

The cytological diagnosis of metaplastic carcinoma is usually challenging, presented with mixed population of malignant ductal cells, spindle cells, and multinucleated giant cells which can be helpful for achieving the correct diagnosis. The sarcomatoid component can be undifferentiated spindle cells or chondrosarcomatous or osteosarcomatous types. Histologically, the different subtypes may be recognized by the evidence of spindle cell component organized in sheets of pleomorphic cells with different lines of differentiation such as angiosarcomatous, leiomyosarcomatous, osteosarcomatous, chondrosarcomatous, or rhabdomyosarcomatous components. The consensus of the working group of WHO was that a descriptive classification system should be adopted (Fig. 3).



Metaplastic Breast Carcinoma, Cytological Findings, Fig. 3 Metaplastic breast carcinoma. Note the squamous differentiation (Papanicolaou stain)

Immunophenotype

Immunohistochemical analysis of metaplastic carcinoma shows that 90% of them are negative for ER, PR, and HER-2. They express keratins 5/6 and 14 and EGFR. The epithelial differentiation requires the use of more than one immunomarker. The usual markers include high-molecular-weight keratins34βe12, keratins 5/6 and 14, and AE1/AE3. Low-molecular-weight keratins are commonly negative. P63 is expressed in at least 90% of metaplastic carcinomas. Markers useful for distinguishing metaplastic carcinoma from phyllodes tumors with sarcomatoid overgrowth include keratins, p63, CD34, and BCL-2.

Molecular Features

Microarray-based gene-expression profiling has shown that metaplastic carcinomas are preferentially classified as basal-like subtype. Some of them, mainly the subtype with spindle cell metaplasia, display transcriptomic features consistent with downregulation of genes usually found in epithelial cells and overexpression of genes found in fibroblasts. They can be classified into a recently described molecular subtype named claudin-low. These tumors have complex genomes, defined by complex patterns of gene copy-number gains and losses similar to those found in other types of triple-negative and basal-like breast cancer. Mutations of the tumor suppressor gene TP53 are found in the majority of these cancers. Loss of CDKN2A (P16) and PTEN are found in a subgroup. Recurrent mutations of PIK3CA and of gene pertaining to the Wnt pathway have been reported as well as EGFR amplification and high polysomy in 10–25%.

Differential Diagnosis

The differential diagnoses include a variety of entity ranging from benign lesions such as nodular fasciitis, fibromatosis, myofibroblastoma, and adenomyoepithelioma and also malignant entity such as “malignant fibrous histiocytoma and pure sarcoma”. The support of immunocytochemistry and electron microscopy may help in the diagnosis of metaplastic carcinoma.

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Metastatic Adenocarcinoma, Effusions

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Synonyms

Adenocarcinoma in serosal fluids

Definition

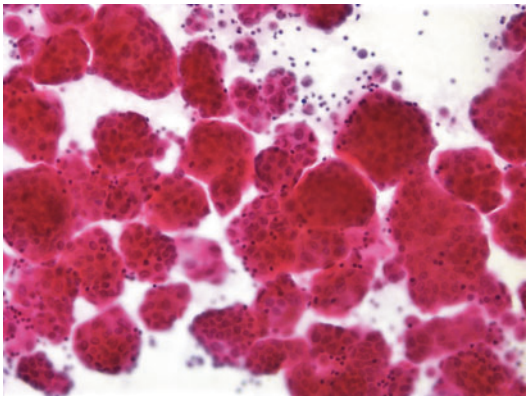
Cytological examination of serous effusions is an efficient, rapid, and noninvasive diagnostic method that is a very important primary diagnostic test for treatment and assessment of prognosis Although the etiology of malignant effusions varies, the most common cause is metastatic carcinomas Among these, adenocarcinomas are by far the most common, especially lung, breast, and ovarian tumors. Malignant mesotheliomas take second place (Dağli et al. 2011; Su et al. 2011).

Such carcinomas often have a tendency to exfoliate in clusters of hyperchromatic pleomorphic large cells; however, in rare occasions, the cells are small and uniform.

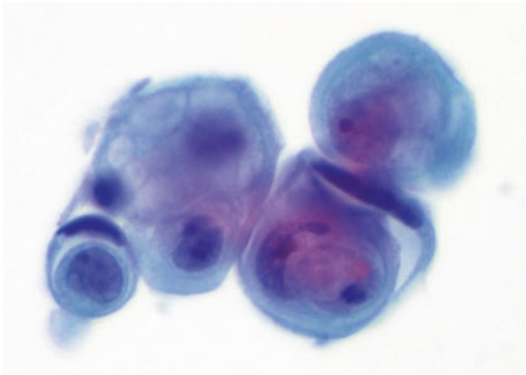
Isolated cells are another manifestation of metastatic adenocarcinomas in effusions. Based on the presenting pattern, i.e., acinar formation, clusters, single cells, cannon balls, linear files, etc., a differential diagnosis can be established. The cells in these cases have a high N/C ratio, hyperchromatic nuclei with irregular contours, and large size. Among the various adenocarcinomas, lung, breast, ovary, and gastrointestinal tract represent the most common primaries (Pereira et al. 2006).

Breast

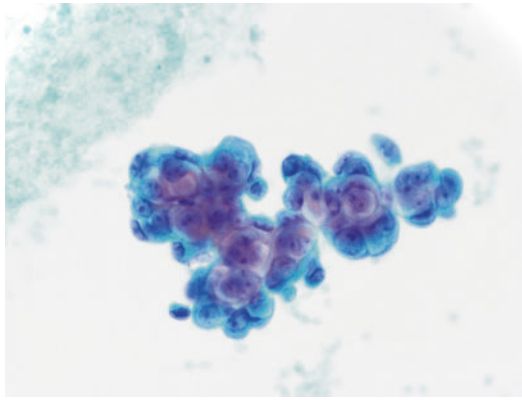
Breast cancer, especially ductal type adenocarcinoma, most commonly manifests as large cohesive spheres, which can also be hollow and are termed "cannon balls" (Fig. 1). Lobular carcinoma of the breast, on the other hand, presents in small chains or as individual tumor cells that resemble histiocytes, mesothelial cells, or signet cells (Pereira et al. 2006). Some studies have shown that a small percentage of ductal adenocarcinomas present as single cells mimicking reactive mesothelium. When presenting as dyscohesive population, frequently, a cell-within-cell pattern can be seen (Fig. 2). It is therefore recommended to utilize a short immunostain panel to exclude breast metastasis in this situation. Signet cells can also be seen in many other types



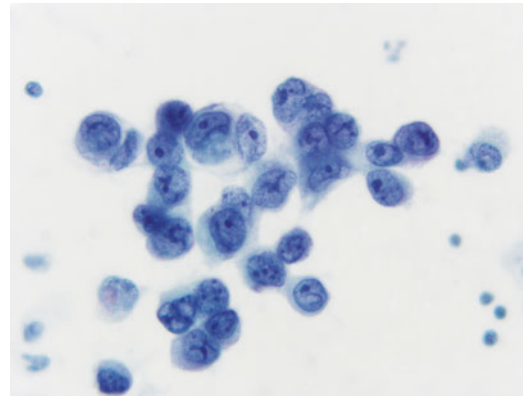
Metastatic Adenocarcinoma, Effusions, Fig. 1 Medium power, Papanicolaou stain demonstrating adenocarcinoma of breast presenting as large rounded clusters "cannon balls" (Pap stain)



Metastatic Adenocarcinoma, Effusions, Fig. 2 High power, Papanicolaou stain demonstrating breast carcinoma presenting as a dyscohesive population of cells frequently displaying "cell-within-cell" arrangement (Pap stain)



Metastatic Adenocarcinoma, Effusions, Fig. 3 Medium power, Papanicolaou stain demonstrating adenocarcinoma of lung presenting as large cell clusters with nuclear atypia (Pap stain)

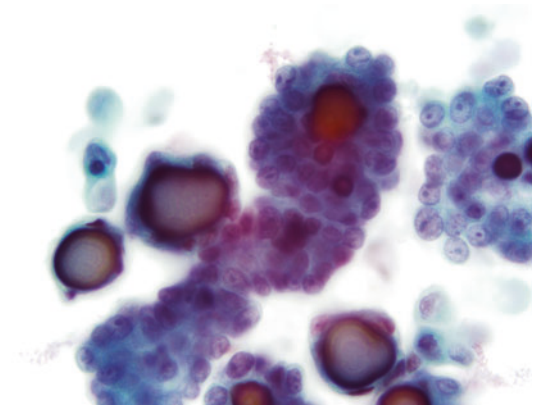


Metastatic Adenocarcinoma, Effusions, Fig. 4 High power, Papanicolaou stain demonstrating adenocarcinoma of lung presenting as dyscohesive population (Pap stain)

of adenocarcinomas, most common of which is gastric adenocarcinoma (Antic et al. 2012).

Lung

The cells are usually clustered in large groups of pleomorphic atypical cells with enlarged nuclei and frothy vacuolated cytoplasm (Fig. 3). Some of these groups may resemble the "cannon balls" seen in breast carcinoma, while others may have a papillary appearance with psammoma bodies. The same cells can also be dispersed singly (Fig. 4) (Michael 2012a).



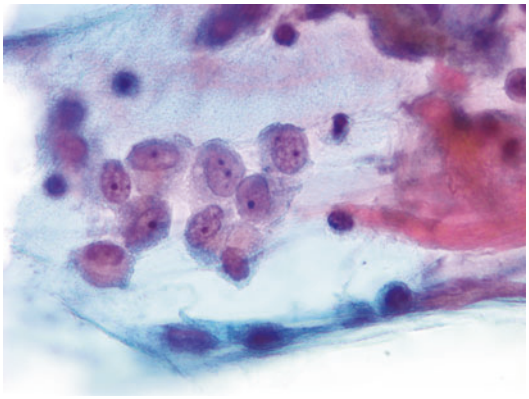
Metastatic Adenocarcinoma, Effusions, Fig. 5 Medium power, Papanicolaou stain demonstrating serous adenocarcinoma of ovary presenting as a large papillary fragment with psammoma bodies (Pap stain)

Müllerian Origin

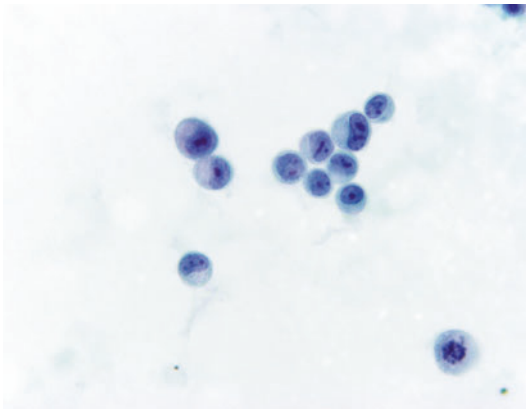
Serous adenocarcinomas of the fallopian tube, ovary, and endometrium present in clusters, which may be papillary, or as isolated cells with marked variation in nuclear size, nuclear hyperchromasia, prominent nucleoli, scant or abundant vacuolated cytoplasm, mitosis, and psammoma bodies (Fig. 5).

Mucinous adenocarcinomas produce abundant extracellular mucin, which when present in the peritoneal cavity is termed "pseudomyxoma peritonei." The tumor cells present in this mucinous effusion are usually sparse and hard to find. They usually lack malignant features, but may sometimes appear atypical (Fig. 6). In some cases, only vacuolated macrophages are present and a definite diagnosis of malignancy cannot be obtained.

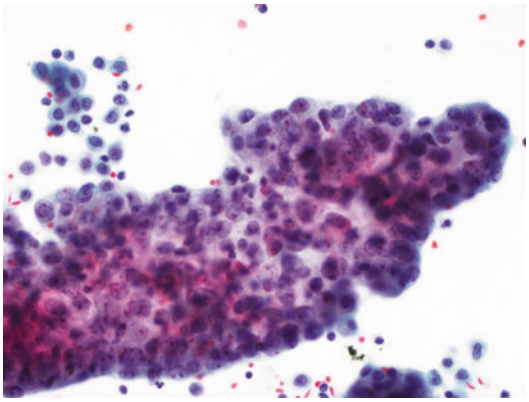
Serous adenocarcinoma of the peritoneum is morphologically indistinguishable from serous carcinoma of the ovary. The malignant cells are isolated or arranged in clusters. There is nuclear hyperchromasia and pleomorphism, with scant or vacuolated cytoplasm, abundant mitosis, and psammoma bodies (Pereira et al. 2006).



Metastatic Adenocarcinoma, Effusions, Fig. 6 High power, Papanicolaou stain demonstrating pseudomyxoma peritonei with few atypical cells in a background of thick mucin and reactive mesothelium (Pap stain)



Metastatic Adenocarcinoma, Effusions, Fig. 8 High power, Papanicolaou stain demonstrating relatively small dyscohesive cells with eccentric atypical nuclei from gastro-esophageal carcinoma (Pap stain)



Metastatic Adenocarcinoma, Effusions, Fig. 7 Medium power, Papanicolaou stain demonstrating a large fragment of highly atypical cells from metastatic colon carcinoma (Pap stain)

Gastrointestinal Tract

Colorectal cancers present with several patterns. They may morphologically resemble acini, with hyperchromatic elongated nuclei and individual cell necrosis. Large clusters, indian filing and cells arranged in small chains have also been described (Fig. 7).

Esophageal adenocarcinoma is characterized by numerous isolated cells, "feathering" at the edges of cellular groups, and haphazard crowded arrangements with some gland formation. Nuclei are enlarged, hyperchromatic, and pleomorphic with irregular nuclear contours and various amounts of vacuolated cytoplasm. Tumor diathesis may be present in the background.

Gastric adenocarcinoma is divided into intestinal type, which mimics esophageal adenocarcinoma, and diffuse type (signet ring cell type). The cytomorphologic features of the signet ring cell type include small groups or isolated cells with vacuolated cytoplasm and crescent-shaped hyperchromatic nuclei (Pereira et al. 2006) (Fig. 8).

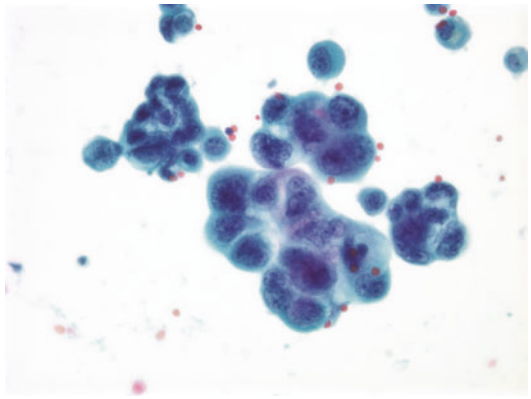
Pancreatic adenocarcinoma presents as clusters or single cells and usually displays a high degree of nuclear atypia (Fig. 9).

Genitourinary Tract

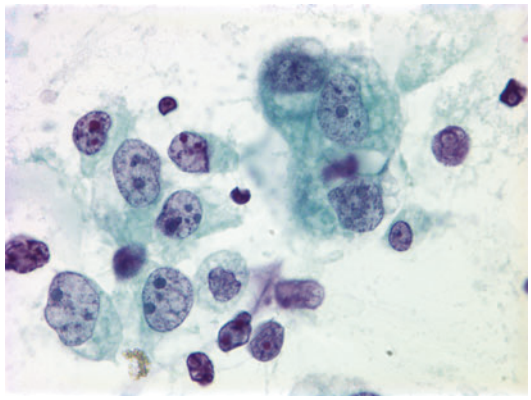
Carcinomas of the genitourinary tract are extremely rare to metastasize in effusions. They tend to produce malignant effusions that are part of a wide range of metastatic diseases.

Clear cell carcinomas of the kidney and female genital tract metastasize to effusions as dyscohesive large cells with vacuolated lacy cytoplasm and prominent nucleoli (Fig. 10).

Prostatic adenocarcinomas may manifest as either isolated cells or as loosely cohesive aggregates of cells with prominent nucleoli. High-grade prostatic adenocarcinoma may sometimes



Metastatic Adenocarcinoma, Effusions, Fig. 9 Medium power, Papanicolaou stain demonstrating clusters of pleomorphic cells from pancreatic adenocarcinoma (Pap stain)



Metastatic Adenocarcinoma, Effusions, Fig. 10 High power, Papanicolaou stain demonstrating a loosely cohesive group with high nuclear grade from metastatic renal cell carcinoma, clear cell type (Pap stain)

resemble small cell carcinoma with abundant nuclear molding, scant cytoplasm, and small isolated cells in chains and clusters (Pereira et al. 2006).

Differential Diagnosis

The main differential diagnosis of metastatic adenocarcinomas in effusions is reactive mesothelial cells or mesothelioma. The presence of a second population of cells that is morphologically distinct from mesothelial cells is the best clue that favors metastatic adenocarcinoma. However, some carcinomas might mimic mesothelial cells and in that case, special stains and immunohistochemistry are most helpful in differentiating the two (Michael 2012b). A positive mucin stain will help shift the diagnosis toward adenocarcinoma. In addition, a panel of immunohistochemical markers for both mesothelial cells and adenocarcinoma can help in distinguishing the two and indicate the site of origin of the metastatic adenocarcinoma. Some authors have compared the sensitivity and specificity of multiple mesothelial immunohistochemical markers and have shown that calretinin and thrombomodulin should be the best for differentiating reactive mesothelial cells/malignant mesothelioma from metastatic adenocarcinomas in serous effusions. Others believe that calretinin and WT-1 should be enough to recognize mesothelial cells.

As for adenocarcinomas, the markers vary with the site of origin. However, it was shown that an antibody panel that consists of E-cadherin and CEA is sufficient to recognize metastatic adenocarcinomas, given their high sensitivity and sensitivity in serous effusions (Su et al. 2011). On the other hand, MOC-31 alone, with a strong membranous staining, was found to be highly sensitive for distinguishing metastatic adenocarcinoma in effusion specimens from reactive mesothelial cells/mesothelioma (Kundu and Krishnamurthy 2011).

Once metastatic adenocarcinoma is favored over reactive mesothelial cells/mesothelioma, the site of origin of the adenocarcinoma will need to be determined.

The distinction between metastatic adenocarcinomas of lung, breast, and ovary in serous effusions can be very difficult since they all can present as tight cell clusters. Immunohistochemical studies with WT1, TTF1, and mCEA antibodies are useful in the differential diagnosis of metastatic adenocarcinomas of lung, breast, and ovary. Studies demonstrate that WT1 stain, while not specific for metastatic carcinoma of ovarian primary, shows a high sensitivity. An immunostaining pattern of positive mCEA and

negative WT1 rules out ovarian adenocarcinoma, raising the possibility of lung or breast adenocarcinomas. A positive TTF1 staining supports the diagnosis of metastatic carcinoma originating from lung rather than breast, while a negative TTF1 favors the diagnosis of a breast primary (Zhu and Michael 2007).

Other markers that can be of help in determining the origin of adenocarcinoma include prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) for prostatic adenocarcinoma. However, it should be noted that less than 50% of metastatic prostate cancers in serous effusions are immunoreactive for these markers (Renshaw et al. 1996).

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Microglandular Hyperplasia, Cytological Findings

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Synonyms

MGH; Microglandular adenosis; Microglandular change; Microglandular endocervical hyperplasia (MEH)

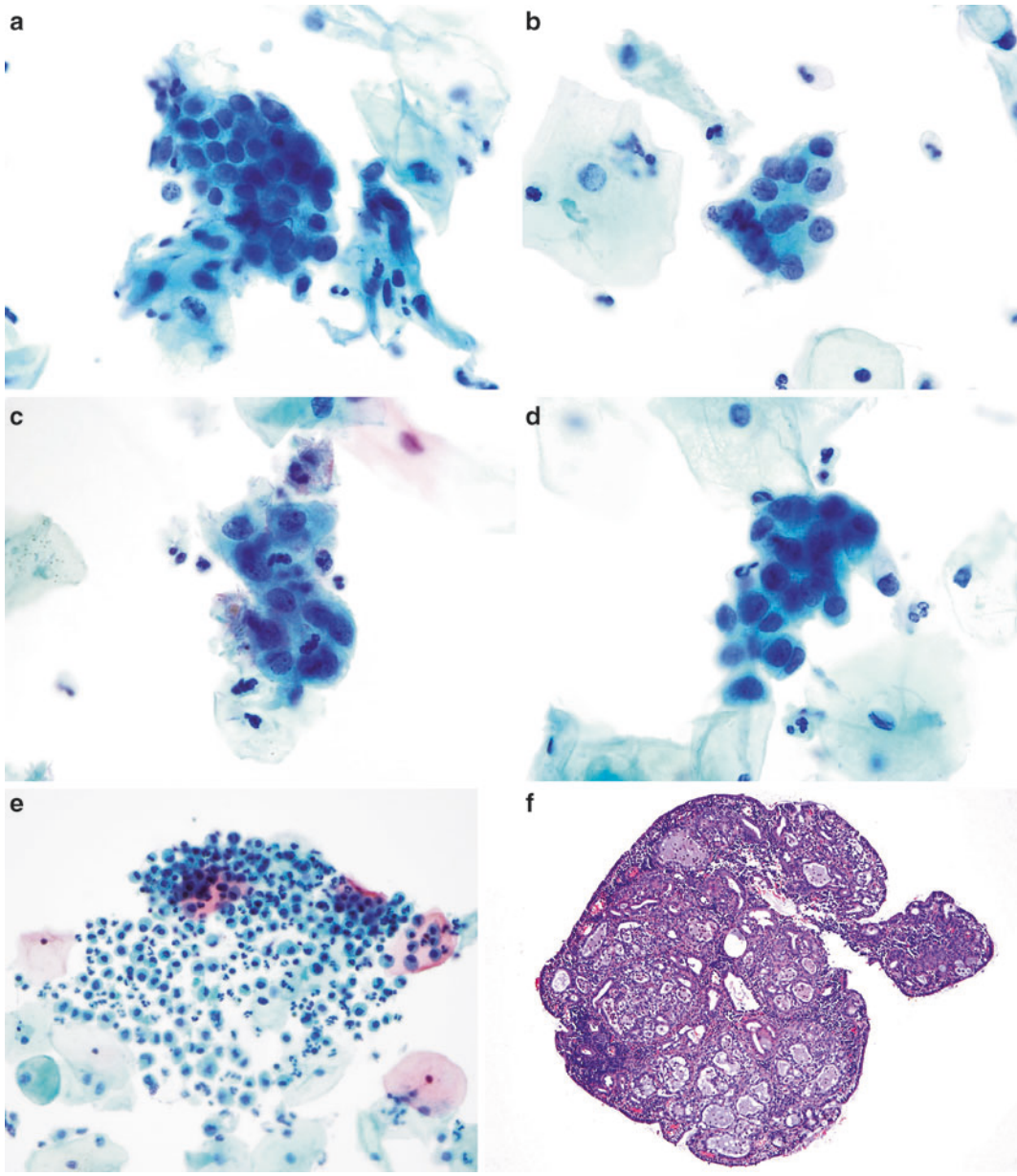
Definition

Microglandular hyperplasia (MGH) is a localized nonneoplastic proliferation of endocervical glands in women of reproductive age. It is associated with oral contraceptive use, pregnancy, and progestins (Fig. 1).

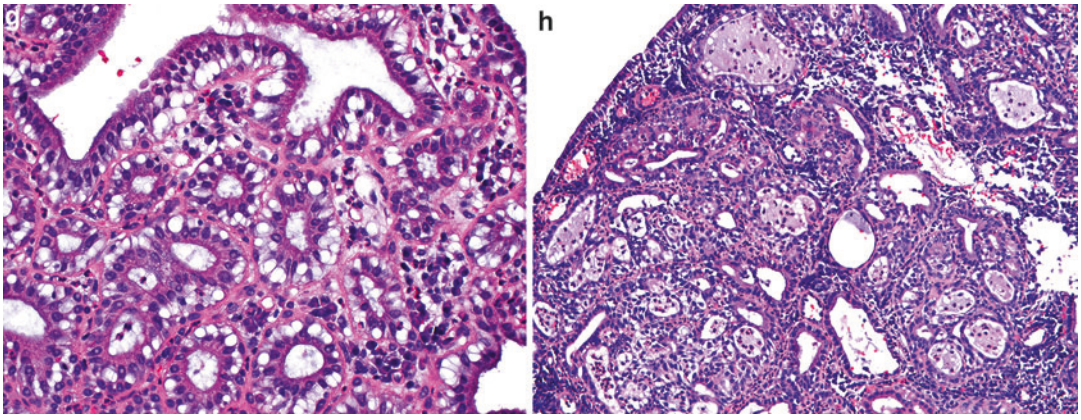
Clinical Features

- **Incidence**
  MGH may be seen in 30% of hysterectomy specimens. Patients can present with complaints of vaginal discharge and abnormal vaginal bleeding but usually they are asymptomatic. MGH is typically an incidental microscopic finding in biopsies or hysterectomy specimens. In the Pap test, atypical glandular cells associated with this condition can be a diagnostic pitfall, as atypia may mimic endocervical or endometrial adenocarcinomas, especially clear cell-type. Histologically, MGH is characterized as a benign tumor-like lesion of the cervix.

- **Site**
  MGH is a nonneoplastic process of the endocervical glands.
- **Age**
  MGH is a relatively common finding among young women (mean age 33.5 years) and is often associated with use of oral contraceptives, postpartum state, and less commonly pregnancy.
- **Treatment**
  MGH is usually an incidental finding and is a nonneoplastic process that does not require treatment. When it presents as a mass and is symptomatic it can be surgically removed.



Microglandular Hyperplasia, Cytological Findings, Fig. 1 (continued)



Microglandular Hyperplasia, Cytological Findings, Fig. 1 (**a**–**d**): MGH shows endocervical glandular cells in two- or three-dimensional cellular clusters. Cytoplasm is scant to abundant and finely vacuolated and in images (**a**) and (**c**) it contains engulfed polymorphonuclear leukocytes. The nuclei are uniform to slightly irregular and oval with fine nuclear chromatin showing distinct nucleoli. In image (**c**) degenerative changes impart a hyperchromatic appearance to nuclei. Note that the cells within clusters in images (**b**–**d**) form microglands or show fenestrations. The nuclear to cytoplasmic ratio is increased (**a**–**d**, ThinPrep, high magnification); (e) Background shows pseudoparakeratosis, a diagnostic hallmark of MGH, characterized by numerous single degenerated endocervical cells with pyknotic nuclei and dense to finely vacuolated basophilic cytoplasm, mimicking squamous parakeratosis; (**f**) Polypoid MGH on surface of the cervix. On clinical presentation, the lesion was protruding through the cervix; (**g**) Florid microglandular endocervical hyperplasia; (**h**) MGH shows complex proliferation of small back-to-back glands lined by cuboidal, columnar, or flattened cells. The nuclei are vesicular, and cytoplasm is eosinophilc with prominent cytoplasmic vacuoles above or below the nuclei (H&E stain, low, medium and high magnification)

- **Outcome**
  MGH is a benign lesion and usually does not require any treatment.

Macroscopy

Single, multiple, polypoid, or sessile lesions which may occasionally be erosive.

Microscopy

The cytological features are not well defined. In some cases, MGH shows clusters of small-to-medium-sized cells or hyperchromatic crowded groups with fenestrations or microacini that appear "atypical." Papillary structures may also be seen. Nuclear enlargement is the most common feature of MGH. The nuclei are uniform, usually oval and overlapping, crowded with fine nuclear chromatin, and with distinct small nucleoli. Degenerative changes may impart a hyperchromatic appearance to nuclei. Cytoplasm is ample, eosinophilic, and vacuolated and contains engulfed polymorphonuclear leukocytes. The nuclear to cytoplasmic ratio is increased. The background may show pseudoparakeratosis, a diagnostic hallmark of MGH. In conventional Pap smears, pseudoparakeratosis is characterized by numerous single degenerated endocervical cells with pyknotic nuclei and orangeophilic finely vacuolated cytoplasm, cells that can mimic squamous parakeratosis. In smears, they appear in linear fashion. In liquid-based preparations, the cytoplasmic eosinophilia or orangeophilia may not be apparent. Plasma cells and inflammatory cells are also seen. In some cases features of classic repair with flat cellular sheets, vesicular nuclei, and prominent nucleoli are noted.

Histologically, MGH shows a complex proliferation of small back-to-back glands lined by cuboidal, columnar, or flattened cells. The nuclei are vesicular and cytoplasm may be eosinophilic with prominent cytoplasmic vacuoles above or

below the nuclei. A solid growth or pseudo-infiltrative pattern may also be seen. In the latter pattern, signet ring cells with focal atypia and hobnail cells within mucin pools (resembling colloid carcinoma) are seen. Occasional mitotic figures, acute and chronic inflammation, and mature or immature squamous metaplasia may also be noted.

Immunocytochemistry

Cytoplasmic vacuoles and lumina formed by a group of cells stain positive for mucicarmine. Cells are usually negative for CEA, CD10, and vimentin. CEA and p16 immunoreactivity are usually seen in usual type of endocervical adenocarcinoma and can be useful tools for differentiating it from MGH.

Differential Diagnosis

The differential diagnosis includes both benign and neoplastic processes. Benign processes include tubal metaplasia and reactive endocervical cells. Tubal metaplasia shows cells similar to MGH, but they are usually arranged in flat sheets and strips of cells with occasional peripheral palisading. Tubal metaplasia is recognized by the presence of cilia and/or terminal bars. Reactive endocervical cells are slightly enlarged with small nucleoli. They are usually arranged as two-dimensional sheets of cells with evenly spaced nuclei. The neoplastic differential diagnosis includes adenocarcinoma in situ (AIS), endocervical adenocarcinoma, endometrial adenocarcinoma, and high-grade squamous intraepithelial lesion (HSIL). Some of the salient features of the neoplastic lesions that help in distinguishing them from MGH include:

- **Endocervical adenocarcinoma (ECA)**: Cytologically, ECA shows background necrosis, architectural and nuclear atypia, and macronucleoli. Histologically an infiltrative pattern is noted; CEA and usually p16 are positive (+).
- **Clear cell carcinoma**: Cytologically, shows background necrosis and architectural and nuclear atypia. Papillary structures, glands, tubules, or small groups lined by hobnail cells may be seen. Nuclei are enlarged, hyperchromatic with macronucleoli, and cytoplasm is vacuolated. Histologically, similar features are noted with marked mitotic activity.
- **Microglandular carcinoma of uterus**: Rarely diagnosed specifically in a Pap smear test. Cytology may be interpreted as adenocarcinoma. Cytologically, background necrosis and endometrioid cells with "dirty" lumina are seen. Histologically, similar features are noted. Vimentin immunostain is positive.
- **HSIL:** HSIL may mimic pseudoparakeratosis of MGH. In HSIL cells are distributed as syncytial fragments and single cells. Nuclei are irregular, hyperchromatic with coarse chromatin, and inconspicuous or small nuclei. Cytoplasm is dense or eosinophilc. Immunostains for p16 and p63 are usually positive.

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Molecular Techniques on Cytology

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Synonyms

Ancillary techniques; Molecular genetics; Molecular pathology

Definition

Any molecular study applied on all types of cytological specimens, namely, gynecology cytology, exfoliative non-gyn cytology, and fine-needle aspirates. Since HPV testing was described in a particular session, here we will focus the applications of molecular techniques in non-gynecological cytology.

Principle

The possibility of using genomic and proteomic studies in small amounts of material obtained, for example, by fine-needle aspiration (FNA), can minimize invasive procedures and allow the monitoring of cancer; in situ PCR, microarray, proteomic, and sequencing (including next-generation sequencing) methodologies are now being validated in some centers but are still not disseminated in the clinical practice (Schmitt et al. 2008; Schmitt and Barroca 2011, 2012; Schmitt and Vielh 2012; Di Lorito and Schmitt 2012; Annaratone et al. 2012). PCR methods are ideal for cytology material, and some applications are detection of gross chromosomal alterations as deletions and translocations or even point mutations in individual genes. Reverse transcription-polymerase chain reaction (RT-PCR) uses cDNA as a template and primers exon sequences to flank rupture points of translocations. PCR applications are centered in diagnosis of solid tumor detecting gene mutations or detecting clone gene rearrangement in lymphoproliferative disorders. However, PCR can also be applied with other methodologies for detection of viral sequences, for example, in situ hybridization (ISH) can also be applied to cytology, permitting, with either fluorescent or chromogenic markers, to detect numerical or structural aberrations of chromosomes.

Methodology (Technical Aspects)

The main challenges for the application of IHC and molecular techniques on cytology are to select the proper test for a limited sample quantity, to avoid jumping blindly applying the protocols that have been set for histology to cytology, and to use appropriate controls for cytological material (Schmitt 2011). Cytology laboratories process different types of material such as fine-needle aspirates (FNAs), cell suspensions, and various types of exfoliative collections. Proper specimen processing is of utmost importance for any ancillary technique. The most commonly used preparations are direct smears, cytospin centrifugations, cell blocks, and liquid-based cytology (LBC) preparations. Direct smears are prepared from FNA material, brushings, or sediment fresh effusions and can be used for molecular studies. After removal of the coverslip with xylene, it is possible to scrape the cells previously stained to an Eppendorf tube, to perform lysis and extract DNA of good quality for PCR or DNA sequencing (Arcila 2012). Cytospin preparations are prepared from cell suspensions in non-fixative solutions such as PBS and RPMI of FNA material or effusions. Air-dried cytospin slides can be stored for many years at −70 °C with excellent DNA preservation. LBC preparation systems are today available in most cytology laboratories. LBC preparations are suitable for preserving cell samples and DNA with sufficient quality to be used in several molecular analyses such as PCR, RFLP, and even sequencing (Longatto-Filho et al. 2009). PCR analysis can be performed directly with freshly collected material from FNA, in

liquid-based cytology samples, or even with cells scraped from FNA slides. In the first condition, the needle should be washed up in ethanol, methanol, or culture media like RPMI. The amount and quality of DNA obtained by FNA for PCR assay does not seem to be a problem, and 50–100 cells are adequate to obtain good PCR results. Monolayer smears are ideal for ISH techniques, and slides with ethanol or air-dried fixed preparations are equally suitable. Cell blocks can be prepared from all types of cytological specimens, except preparations with low cellularity such as cerebrospinal fluids. The morphology of cell blocks is identical to that seen in histological specimens and therefore familiar to most pathologists. The technique, which is available in most laboratories, is relatively time-consuming, and the cost is comparable to that of the cytospin technique. Since most of molecular techniques are now standardized for paraffin-embedded tissues, they can apply directly to cell-block preparations with excellent results. Controls are another important concern when we use cytological material. The basis of a good and valid technique is a correct choice of positive and negative controls verifying the sensitivity and specificity of the technique. To preserve a good quality material, maintaining morphology and nucleic acid integrity must be another priority. As mentioned before, liquid-based cytology preparations are suitable for preserving cell samples and DNA with sufficient quality to be used in several molecular analyses. ISH is one of the techniques where the interpretation of the molecular changes is simultaneous with the morphological control. Chromosomal translocations are one of the most common genetic alterations studied in pathology. Several methods exist to evaluate them, including conventional cytogenetics, RT-PCR, and ISH. ISH offers several advantages over conventional cytogenetics. Its main advantage is that nondividing (interphase) nuclei can be evaluated, making it unnecessary to evaluate the neoplastic cells in culture. This allows a retrospective analysis of alcohol-fixed smears or formalin-fixed, paraffin-embedded cell blocks. ISH is especially advantageous in small samples, in which it may not be possible to submit tissue for cytogenetics. ISH also offers several benefits over RT-PCR. For a given translocation with multiple breakpoints, multiple primers are necessary to evaluate all possible fusion transcripts, whereas, with FISH, this can be achieved by using break-apart probes (with split signals reflecting the presence of gene fusion) or dual-fusion translocation probes (with fusion of signals indicating the presence of gene fusion). Therefore, one set of FISH probes can identify essentially all known breakpoints of a given translocation, resulting in increased sensitivity. In addition to the advantages of evaluating multiple breakpoints, FISH also is helpful in situations in which one translocation partner is constant but the second translocation partner changes. Rapid advances in sequencing technologies have brought the cost of sequencing DNA down. Traditional sequencing technologies rely on a labor- and resource-intensive process of cloning fragments of sample DNA into bacteria, selecting and growing the clones, and then sequencing purified copies of a single fragment. Each traditional sequencing reaction yields approximately 1 Kb (1,000 bp) of DNA sequence; typically about 100 reactions are run in parallel. In contrast, next-generation sequencing technologies take advantage of miniaturization and automation to sequence hundreds of thousands to millions of DNA fragments in parallel using extremely small amounts of chemical reagents per reaction (Di Lorito and Schmitt 2012). A single sequencing run with next-generation technologies can yield more than 100 Gb (100 billion bp) of DNA sequence in a matter of hours or days, depending on the particular technology used. On the horizon are "$1,000 genome" technologies that further refine sequencing by allowing direct sequencing of individual DNA molecules. Such refinements have the potential to eliminate the need for sample amplification, further reduce the use of chemical reagents, and produce highly accurate sequence data more rapidly. Next-generation DNA sequencing is revolutionizing cancer genomics. Whole genome resequencing of tumors is within our reach. High-quality reference genome sequences provide us prospect to study genetic variation on an unprecedented scale. Genes, genomic regions,

and whole genomes can be resequenced, aligned to appropriate references, and genetic peculiarity between individuals can be detected. There are many applications of this high-throughput method in cytological material. The possibility to detect many genetic alterations in one "shot" opens avenues to study in cytological material a panel of mutation, many of them can be "druggable" target. The demonstration that during lung cancer evolution there are several genotypic and phenotypical changes that can imply in acquisition of drug resistance and in some cases later loss of this mutation and development of drug response is another opportunity to apply this technology in cytology to follow these patients.

Quality Aspects

There is a wide range of molecular techniques such as ISH, PCR, RT-PCR, microarrays, and sequencing that can be used to study DNA and mRNA alterations in exfoliated cells from serous fluids or obtained by cytobrush as well as in cells obtained from FNA. However, it is outside the scope of this text to review technical details from all these techniques. The introduction of these molecular techniques raises an important point: how to preserve good quality material maintaining cellular morphology and DNA/RNA integrity. As stated before LBC material is suitable for the preservation of cell samples and DNA with sufficient quality to be used in several molecular techniques. DNA/RNA is stable from period of up to 3 years in this type of media (Longatto-Filho et al. 2009; Schmitt 2011). However, the horizons for the use of molecular techniques on cytological specimens have been expanded, with studies showing the applicability of these techniques to archival FNA samples, which are extremely useful in situations in which the diagnostic material may be limited to certain slides. The application of protocols that test the quality and quantity of storage DNA is a rule of good practice (Pang et al. 2011; Schmitt 2011). However, most of the standardized high-throughput molecular methods for the measurement of gene expression, such as gene expression profiling, require a sufficient quantity of high-quality RNA obtained from fresh or frozen tissues. Annaratone et al. (2012) showed that the leftover, residual material from the smearing procedures of FNA samples contains an important source of fresh cells, suitable for banking and appropriate for molecular analysis of small non-palpable breast lesions. The results demonstrate that RNA can be successfully extracted from the FNA leftovers for gene expression profiling using oligonucleotide microarrays. Clusters generated by global expression profiles partitioned samples in well-distinguished subgroups that overlapped with clusters obtained using "biological scores" (cytohistologic variables) and differed from clusters based on "technical scores" (RNA/complementary RNA/microarray quality). Morphological control may be one of the most crucial issues in the application of molecular techniques to cytology. Pang et al. (2011) have shown the value of the enrichment of tumor cells in microdissection and how this should maintain the analytical sensitivity of the test, which, in their case, was 10% of malignant cells in a background of 90% of nonneoplastic cells. The role of the cytopathologist is mandatory in the collection and selection of cells. For sequencing, in our laboratory we set at 20% of tumor cells present in the sample with the method sensitivity. We observed that the global rate detection of mutations increases from 26% to 40% when we consider only specimens with more than 20% of tumor cells.

Applications

There are many possible applications of the use of ancillary techniques in different organs and cytological material. In this review we will focus in the most frequent applications and in the organs that the author has more practical experience.

Lung Cytology

The most important molecular discoveries associated with lung cancer are related to the use of selective TKIs targeting EGFR mutation and the EML4–ALK fusion gene. Today, all pulmonary

adenocarcinomas must be tested routinely for the presence of EGFR or KRAS exclusive mutation, and if both these markers are negative, it should be tested for EML4–ALK translocation (Travis et al. 2011). Molecular detection of EGFR mutation is at this moment the most accurate method to select these patients. A published meta-analysis showed that different molecular techniques, such as FISH, quantitative PCR, and direct sequencing, using fresh cells, scraped cells from archival slides and cell blocks, have similar or higher accuracy and sensitivity than surgical specimens (Da Cunha Santos et al. 2011). The most common EGFR mutations are small in-frame deletions in exon 19 and a point mutation (L858R) in exon 21. Together these two mutations account for 90% of all EGFR mutations in non-small cell lung cancer (NSCLC). The screening of EGFR mutations is not only to select patients for treatment but also to detect the resistance mutation (a substitution T790M in exon 20 appears in about half of all patients with acquired resistance to TKI) (Sequist et al. 2011). Since these mutations can appear during the progression of the tumor, cytology is a good alternative to follow the patients and using techniques such as deep sequencing to study all genomic variations. In 30% of lung adenocarcinomas (ADCs) and 5% of squamous cell carcinomas (SCC), KRAS mutations are found. These two types of molecular alterations, KRAS and EGFR, are mutually exclusive, and patients with the KRAS mutation do not benefit from the abovementioned therapy. The fusion of the ALK gene with echinoderm microtubule-associated protein-like 4 (EML4) is detected in 3–5% of the NSCLC (Savic and Bubendorf 2012). ALK and EML4 are both located in the short arm of chromosome 2 separated by 12 Mb and are oriented in opposite 5–3′ directions. Fusion of ALK with EML4 (or rarely with other fusion partners such as TFG or KIF5B) leads to constitutive activation of the ALK kinase. Treatment of patients with EML4–ALK fusion-positive NSCLC by the small-molecule kinase inhibitor crizotinib revealed a response rate of 50–60% and improved overall survival. Recent recommendations suggest systematic ALK testing in advanced, preferably EGFR/KRAS wild-type non-squamous NSCLC (Savic and Bubendorf 2012). In the FDA-approved Vysis ALK FISH probe kit (Abbott Molecular), the 3′ and 5′ ends of the ALK gene are differentially labeled with orange and green fluorescence probes. In benign cells, the two signals are close together. In case of anEML4–ALK gene fusion by inversion alone, which makes 60–70% of all ALK rearrangements, the red and green signals are separated from each other ("break-apart" probe). In another 30–40%, gene fusion occurs by interstitial deletion together with an inversion of the remaining DNA stretch that includes EML4. This results in single orange signals without corresponding green signals in addition to fused signal (Savic and Bubendorf 2012). For the analysis, the previously stained cytological slides should first be evaluated for suitability and tumor cell content analysis, and the area for hybridization should be marked. A total of 50 nuclei need to be scored using a ×60–100 oil immersion objective lens. Cells are considered positive when (a) at least one set of orange and green signals is two or more signal diameters apart (indicating gene fusion by inversion) or (b) there is a single orange signal without a corresponding green signal in addition to fused (normal) signals (indicating gene fusion by inversion and deletion). A single green signal without a corresponding orange signal is considered negative. According to manufacturer instructions (Abbott Molecular), a sample is considered negative for ALK rearrangement if there are less than 5 positive cells (less than 10%) and positive if there are more than 25 positive cells (more than 50%). A sample is considered equivocal if 5–25 cells (10–50%) are positive. In this case, a second reader should evaluate the slide. If the average of the two readings contains at least 15% positive cells, the sample is considered positive for ALK rearrangement (Savic and Bubendorf 2012).

Serous Effusions

The molecular techniques applied in effusions are based on detection of genetic alterations representative of malignancy, using karyotyping, ISH, PCR, among others. The expression of telomerase is an example of one of these

applications. Malignant cells are distinguished from benign cells by their immortality, due to the overexpression of telomerase and consequent telomere maintenance. This overexpression of telomerase may be measured by the technique "TRAP (telomeric repeat amplification)," which increases the sensitivity of cytologic screening at detection of neoplastic cells. Recently, Savic et al. (2010) demonstrated that using the FISH technique to detect the deletion of the chromosomal region 9p21 allows distinguishing mesotheliomas of reactive mesothelial cells on cytology of serous effusions with a sensitivity of 79% and specificity of 100%. The detection of several chromosomal alterations, for example, using commercial probes which detect numerical abnormalities in chromosomes 3, 7, and 17 and of locus 9p21 (gene p16INK4a), has proven useful in the detection of malignant cells in effusions, with a specificity close to 100% in cases of doubtful cytology. In effusions of lung cancer, EGFR mutation and ALK translocations can be also detected for therapeutic purposes.

Urine Cytology

Among all the potential techniques that can be used to detect molecular alterations in urine, only the UroVysion multiprobe FISH (U-FISH) is used on clinical practice (Bubendorf 2011). Urinary cytology is highly specific for the diagnosis of high-grade urothelial carcinoma and carcinoma in situ, but it has a low sensitivity for low-grade, noninvasive urothelial tumors. The U-FISH consists of fluorescently labeled DNA probes to detect increased copy numbers of the chromosomes 3, 7, and 17 and deletion of 9p21, the site of p16 gene. Multiple studies have shown that FISH on voided urine or washing specimens has more sensitivity over cytology in different circumstances. There are particular situations where this test is indicated: equivocal cytological findings, control after intravesical BCG treatment, upper urinary tract cytology, surveillance after transurethral resection, and hematuria in patients with increased risk of cancer. Patients with negative or equivocal cytology but with FISH positive have a risk of 50–80% for developing a recurrent urothelial carcinoma (Volpe et al. 2008). However, despite literature demonstrating the excellent performance of this method to overcome the limitations of cytology, it is important to emphasize that molecular biology should be always used wisely and in well-defined occasions because U-FISH is rewarding in atypical cytological findings but redundant and not cost-effective to detect high-grade lesions. A word of caution should be mentioned concerning the interpretation of the test. Chromosomal numerical abnormalities are not restricted to malignancy but can also occur in benign cells. In particular, tetraploidy with a balanced duplication of the whole genome can succeed in nonneoplastic conditions of the bladder (Bubendorf 2011). Most of these cells can easily be diagnosed as activated umbrella cells by morphology. Therefore, FISH should not be diagnosed as positive, solely based on cells with a tetraploid pattern. In contrast, unbalanced numerical changes of one or more chromosomes (e.g., 2, 3, 5) or loss of 9p21 is virtually specific for neoplasia in bladder cytology.

Thyroid Cytology

The finding that some thyroid lesions are associated with specific genetic alterations raises the possibility of improving FNA cytology diagnoses with access to molecular techniques. Papillary thyroid carcinomas (PTCs) frequently are diploid lesions and can display non-overlapping mutations of the genes v-raf murine sarcoma viral oncogene homologB1 (BRAF), ret proto-oncogene (RET), and RAS in 46–75%, 3–85%, and 0–21% of cases, respectively (Eszlinger and Paschkea 2010). Although the prevalence of these mutations varies among the published series, their presence is very specific and indicates the existence of malignancy. The follicular variant of PTC has a mutational framework that differs slightly from other PTCs. A distinct BRAF mutation is observed ([K601E]) in 7% of tumors, RAS mutations are more frequent (approximately 25% of tumors), and they can have a PAX8–peroxisome proliferator-activated receptor gamma (PPARgamma) mutation (38% of tumors). Follicular neoplasia is effectively an aneuploid lesion and has a high

prevalence of RAS and PAX8–PPARgamma gene mutations (33% vs. 45%, respectively, in follicular adenomas vs. follicular carcinomas). Characteristic follicular neoplasia mutations are less specific than those described in PTCs and do not necessarily point to malignancy. Somatic rearrangements of RET are considered specific of PTCs. There are two types of mutations in RET/PTC most commonly related to PTCs: RET/PTC1 and RET/PTC3. RET/PTC1 prevails in classic and diffuse sclerosing variants, whereas RET/PTC3 predominates in solid and follicular variants of PTC. Nikiforov et al. (2011) demonstrated that using a panel of markers to detect BRAF, RAS, PAX8–PPAR, and RET/PTC mutations increased the overall accuracy of FNA. When indeterminate aspirates were analyzed for the presence of BRAF and RAS mutations and for RET/PTC and PAX8–PPARgamma gene rearrangements, mutations were found in 16% of cases. These genetic markers have high specificity and a high positive predictive value and therefore identify which indeterminate nodules are malignant. Marker positivity can lead to a recommendation for total thyroidectomy rather than for hemithyroidectomy or watchful waiting. Although useful, these markers have limited sensitivity and a limited negative predictive value and therefore fail to detect more than 33% of cancer. Thus, currently available molecular markers fail to rule out cancer with sufficient certainty to avoid surgery in most patients with indeterminate nodules. Recently, a RNA-based test where the cells are aspirated using two passes and placed into a preservative solution was developed (Afirma gene expression classifier [AGEC], Veracyte, San Francisco, CA) for the preoperative identification of benign thyroid nodules whose cytology is indeterminate. The microarray assay uses an algorithm based on the expression of 167 genes to classify aspirated material as either benign or suspicious. The assay classifies nodules as either benign (>95%NPV) or suspicious for malignancy (>50% risk for malignancy). A large prospective validation study of the AGEC showed an overall NPV of 94–95% for AUS/FLUS and follicular/Hurthle cell neoplasm subtypes (Alexander et al. 2012). The NPV for cytology that proved suspicious for malignancy was lower, however (85%), indicating that the AGEC should not be used to avoid a surgery in this cytological subtype, although it may be helpful in the decision to consider obectomy versus total thyroidectomy. However, a repeat FNA alone can accurately reclassify more than 50% of nodules in the category AUS/FLUS as benign. In addition, 1–2% of repeat FNA from the AUS/FLUS category are diagnosed as "suspicious for malignancy" or higher and would go directly to surgery without the need for additional testing. Further progress in molecular diagnostics of thyroid nodules is likely to be achieved taking advantage of the availability of new technologies, such as next-generation sequencing. Such approach will allow to expand the currently existing panel by adding multiple genetic alterations known to occur in thyroid cancer. This will improve the accuracy of cancer diagnosis in thyroid nodules with indeterminate cytology.

Gastrointestinal Tumors (Including Pancreas) Cytology

Endoscopic ultrasound-guided FNA biopsy (EUS-FNA) has been used increasingly for the assessment of diverse intra-abdominal tumors. EUS-FNA not only allows for a careful representation of both extramural and intramural structures of the gastrointestinal tract but also permits tissue sampling from tumors in these locations. In this section, we discuss the applications of molecular cytopathology to refine the diagnosis of pancreatic tumors and to set a precise treatment in gastrointestinal stromal tumors (GISTs). For practical purposes, we can divide pancreatic tumors into solid and cystic lesions. In general, the diagnosis of ductal ADC is straightforward; however, in some situations, additional markers can be helpful. Recently it demonstrated the use of UroVysion fluorescence in situ hybridization (U-FISH) (Abbott Molecular Inc, Des Plaines, Illinois) to improve the diagnosis of pancreatic malignancy in FNA material (Henkes et al. 2013). Like urinary samples, a positive pancreatic U-FISH result was defined as a significant number of cells with polysomy, trisomy, or loss of the 9p21 locus. In particular, U-FISH test results that

are polysomic are highly specific for a pancreatic adenocarcinoma. The high specificity of U-FISH may help increase the ability of the pathologist to make a definitive diagnosis for patients who have clinical, imaging, and cytological features that all suggest malignancy. A polysomic U-FISH test result in an FNA sample with atypical cells is virtually diagnostic of malignancy. A negative U-FISH result does not rule out malignancy but may prompt the search for other causes of a sonographic or radiographic mass. The most common cause for a negative U-FISH result for a patient with pancreatic adenocarcinoma was insufficient numbers of malignant cells for the test. The diagnosis of pancreatic cysts requires a multidisciplinary approach (Pitman et al. 2010). Imaging plays an essential role in identifying cystic morphologic details and conducting the screening between probably benign and probably malignant cysts. The introduction of EUS-FNA with rapid on-site cytologic evaluation gave cytology a great role in the management of pancreatic cysts. Supported by imaging techniques, the role of the cytopathologist is first to discriminate pseudocysts from neoplastic cysts and, then, to separate mucinous cysts from serous cysts, avoiding a possible misdiagnosis with contaminant stomach or duodenum epithelia. The cystic evaluation of amylase CEA and in selected cases of KRAS mutations or DNA content can be of great aid, separating benign from malignant lesions. KRAS mutations or loss of heterozygosity in >2 loci is associated with mucinous cysts and helps predict malignancy. CEA is a good marker for distinguishing between mucinous and nonmucinous cysts, but it does not distinguish benign from malignant lesions (Khalid et al. 2009). In negative KRAS samples, the CEA level can capture almost 70% of cases. In scanty samples (<1 mL of fluid), it may be tempting to rely too heavily on the detection of KRAS mutations; however, recent studies have demonstrated that molecular analysis has lower performance in predicting mucinous cysts than CEA analysis. The ideal procedure stems from the combined use of both techniques (Schmitt and Barroca 2012). GISTs are characterized in >80% of cases by a mutation of the CKIT proto-oncogene with subsequent autonomous activation of the tyrosine kinase receptor that induces tumor development and proliferation. In 5% of GISTs, there is also an active mutation of the platelet-derived growth factor receptor (PDGFRA) gene. These mutations are mutually exclusive and represent two different alternative oncogenic events that lead to similar biologic consequences. Imatinib is first-line therapy for patients with advanced GISTs. It acts as an inhibitor of the tyrosine kinase receptor KIT and PDGFRA. The most common mutation, and also the most responsive to TKIs, is on exon 11 (juxtamembrane domain) of CKIT. The other involved mutations, which are less responsive in GISTs, are related to exon 9 (extracellular domain), exon 13, and exon 17 (tyrosine kinase domain). Also, PDGFRA mutations vary according to the exon involved. Exon 18 (tyrosine kinase domain) is much more responsive to imatinib than exon 12 (juxtamembrane domain). This is where FNA has its main molecular application in selecting patients for therapy. Our group previously demonstrated the feasibility of performing molecular analysis of CKIT and PDGFRA genes in cytological material obtained by EUS-FNA from 85 patients with intramural gastrointestinal mesenchymal tumors (Gomes et al. 2007). This and other studies demonstrated that a precise preoperative diagnosis of GIST can be achieved with EUS-FNA, and the detection of mutations in cytological samples also allows the prediction of therapeutic response, enabling greater efficiency in the use of neoadjuvant therapy.

Soft Tissue Cytology

Many soft tissue tumors harbor characteristic translocations or amplification of gene regions that can be assessed by molecular methods and that have a great impact on the cytological diagnosis of these entities (Schmitt and Barroca 2011, 2012). In this field, one should make a special reference to the small blue round-cell tumor group (SBRCT). Tumors such as ES/PNET, alveolar rhabdomyosarcoma, synovial sarcoma (undifferentiated type), mesenchymal chondrosarcoma, and desmoplastic small round-cell tumor are characterized by specific molecular

changes. In more than 85% of Ewing's family tumors (EFTs), there is a specific tumor-associated translocation t(11;22) (q24; q12), juxtaposing the EWS gene on chromosome 22 with the FLI1 gene on chromosome 1. The identification of EWS/ETS fusion in problematic cases is a helpful tool for cytological differential diagnosis as recently demonstrated in a series of 50 patients (Klijanienko et al. 2012). Alveolar rhabdomyosarcoma (ARMS) is also characterized by specific translocations. Two forms of translocation have been associated with ARMS: t(2;13) (q35;q14) in 70% of the cases and t(1;13) (p36; q14) in 10–20% of the cases. These translocations besides determining the cytological diagnosis reflect a hint to prognosis. ARMS with t(1;13) were found to have better prognosis than those with t(2;13) (q35;q14). Undifferentiated synovial sarcoma (SS) composed of primitive small round cells occurs in 15% of the cases and represents a challenge in the differential cytological diagnosis with all the other small round-cell tumors. The detection through RT-PCR of a reciprocal translocation t(X;18) (p11.2;q11.2) or the rearrangement of SYT gene by FISH is seen in more than 90% of the cases and is highly specific of SS being essential to achieve a correct diagnosis in problematic cases (Tanas et al. 2010). Desmoplastic small round-cell tumor (DSRCT) is a highly malignant mesenchymal neoplasm whose cytological smears resemble those of all other malignant small round-cell tumors. Cytogenetically DSRCT is characterized by a reciprocal translocation, t(11;22) (p13;q12) (Schmitt and Barroca 2012; Tanas et al. 2010). Low-grade myxoid neoplasms represent another group of soft tissue tumors where the cytological aspects are not enough to achieve a correct diagnosis. The differential diagnosis in this group includes low-grade fibromyxoid sarcoma, myxoma, myxofibrosarcoma, and myxoid liposarcoma. FISH probe set for FUS gene region (16p11) will detect the split apart in either t(7,16) or t (11,16) in low-grade fibromyxoid sarcoma or t (12,16) in myxoid liposarcoma. The FISH test for DDIT3 gene region (12q13) which is split apart in t(12,16) and t(12,22) is extremely useful and specific in the diagnosis of myxoid and round-cell liposarcoma (Tanas et al. 2010). The diagnostic utility of MDM2 amplification detection in FNA of dedifferentiated liposarcoma has been recently demonstrated (Al-Maghraby et al. 2010). Although rare, sometimes the problem of differential diagnosis between primary clear cell sarcoma and metastatic malignant melanoma can be a fact in FNA. Morphology and IHC can be identical, and in these cases, besides clinical data, the detection of EWSR1 by FISH is diagnostic of clear cell sarcoma which harbors a t(12,22), resulting in rearrangement of the EWSR1 gene region (Tanas et al. 2010). In neuroblastomas, cytology and genetics can work together to define the biological behavior and treatment of these tumors. Neuroblastoma treatment settles in preestablished risk groups, defining different biologic behavior groups and ensuing different therapeutic strategies. These groups combine age at diagnosis, clinical stage (INSS, International Neuroblastoma Staging System), histology (INPC, International Neuroblastoma Pathology Classification), MYCN oncogene status, deletion of the short arm of chromosome 1, and DNA index. DNA content and mainly *N*-myc amplification have been established as independent prognostic markers in neuroblastomas. At the time of diagnosis, material should be kept to evaluate DNA content and also to perform FISH or Southern blotting analysis in order to detect *N*-myc amplification. Several reports, including from our group, indicate that FNA of neuroblastic tumors allows the collection of enough material to evaluate the DNA content of the tumor and detection *N*-myc amplification (Barroca et al. 2001).

Metastatic Cancer Cytology

FNA can be a safe, trustable, and cheaper alternative to obtain cells from metastatic sites to study cell characteristics (Schmitt and Vielh 2012). An important field to use molecular assessment is metastatic breast cancer (MBC). MBC is usually diagnosed by a combination of clinical and imaging findings. Once diagnosed, the choice of systemic therapy is based on the ER, PR, and HER2 status from the patient's primary tumor. Biopsy of suspected metastatic lesions is rarely

done. However, tumor characteristics can change, and discrepancies between primary and metastasis are described with variations until 30% for the hormonal receptors and 5–10% in HER2 (Amir et al. 2011). Wilking et al. (2011), using FISH for HER2 in FNA samples from metastatic sites of breast cancer, showed an intra-patient agreement in HER2 status of 76% and a disagreement of 10%. The unstable status for HER2 in breast cancer is clinically significant and should motivate more frequent testing of recurrences. In our own experience, we observed 15% of disagreement between HER2 assessment in primary and respective metastases of breast cancer, using FISH on FNA material. So, in conclusion, FNA is a less traumatic method that provides a good source of breast cancer cells, including from metastatic sites, to perform ISH for HER-2 with excellent quality of technique and preservation of integrity of the nuclei and signals. Cetuximab and panitumumab are EGFR monoclonal antibodies that, either alone or in combination with chemotherapy agents, have been proven effective in the treatment of patients with metastatic colorectal cancer (Schmitt and Barroca 2012; Schmitt and Vielh 2012). KRAS gene mutations have been pointed out as possible molecular diagnostic markers for predicting the sensitivity of tumors to anti-EGFR therapeutics. Patients with tumors that harbor KRAS mutations have a lower drug response rate; however, a significant percentage of patients with KRAS–WT tumors also do not respond to anti-EGFR drugs. One of the many possible reasons for this is the discrepancy between KRAS status in the primary tumor and in metastasis. In a series of 250 patients with sporadic colon cancer who were analyzed in our institution, we detected 17% discordance between mutation status in KRAS and BRAF genes when comparing primary and metastatic samples (Oliveira et al. 2007). These results are of fundamental importance, because they may represent one of the resistance mechanisms interfering with the response of patients with KRAS–WT metastatic colorectal cancer to anti-EGFR monoclonal antibody therapy. Moreover, it is estimated that 20% of the target patient population will present with metastatic disease, and there will not be access to archive material from the primary tumor. In both situations, FNA material obtained from metastatic sites is a good, safe, and cheap alternative for determining KRAS status in metastatic colon cancer (Pang et al. 2011). Melanoma is a tumor that is frequently diagnosed in a metastatic site by FNA. These patients have a dismal prognosis (8–10-month median overall survival). In the last three decades, there were no advances in therapy. However, it was recently demonstrated that 50% of melanoma cases have BRAF mutation and recently inhibitors of BRAF were described and tested with success. So, it is mandatory to test metastatic melanoma cells for BRAF mutations, and FNA is a suitable method to obtain these cells. Hookim et al. (2012) showed that the use of cytological direct smears proved to be a robust and reliable methodology for molecular testing. In this study mutation testing was successfully performed using cellular material microdissected from archived, Diff-Quick-stained, decoverslipped smears. Overall, BRAF mutations were observed at the expected frequency because 53% of the tumors analyzed harbored mutations in BRAF. The majority of the mutations resulted in the V600E substitution, and a minority of tumors exhibited the V600K substitution. This is consistent with prior observations that the most common BRAF mutation in melanoma is V600E followed by V600K. Another challenge in metastatic tumors is the fact that during cancer evolution there are genotypic changes that can imply in modification of drug response, for example, with acquisition of drug resistance (Sequist et al. 2011). As mentioned before, the possibility to use NGS technology in cytological material will allow us to follow all these modifications, since FNA is an easy and non-harmful technique to obtain cells from metastatic sites.

Conclusions

Cytology offers a suitable alternative to biopsy in a variety of clinical settings, and there are many studies showing the possibility of using cytological specimens to study response to therapy and

evaluation of morphological changes of tumor cells with minimal discomfort for the patients. Because therapies are now being directed toward individual molecular targets, the challenge for using this technology in cytology is to increase standardization of pre-analytical and analytical methods. High-quality DNA/RNA in sufficient quantity may be obtained from cytological methods allowing molecular studies even in small tumors. Today it is essential for the pathologists, to keep good material, to validate in large-scale molecular studies in cytological samples, and to control the cases morphologically.

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Mucinous Breast Carcinoma, Cytological Findings

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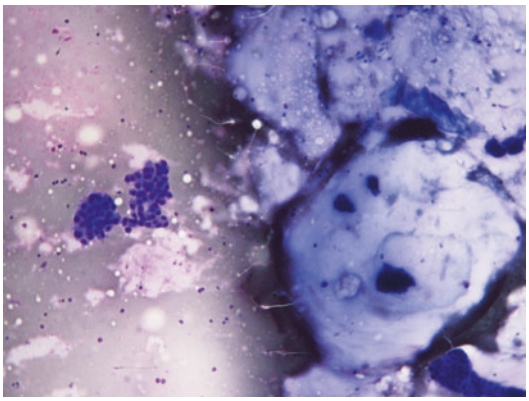
Synonyms

Colloid carcinoma; Gelatinous carcinoma; Mucoid breast cancer



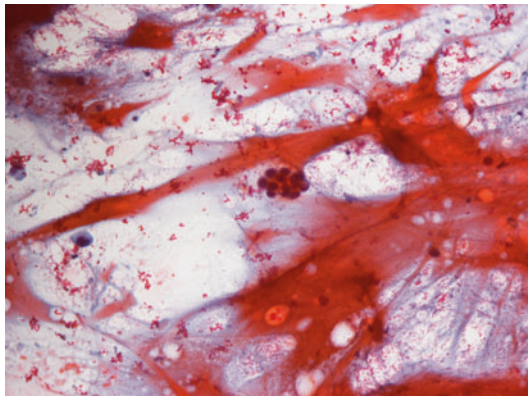
Mucinous Breast Carcinoma, Cytological Findings, Fig. 1 Mucinous breast carcinoma. Groups of neoplastic cells in the middle of abundant mucin (Giemsa stain)

Definition

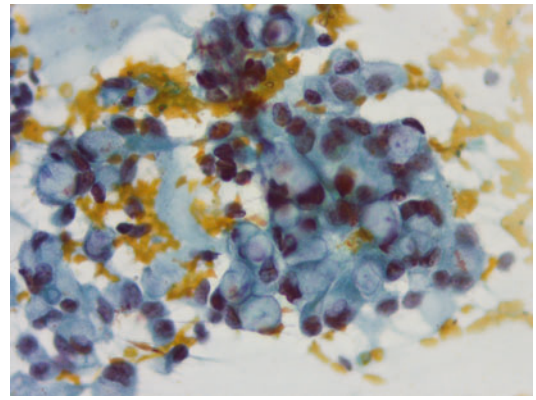
It is a variant of an invasive carcinoma defined by neoplastic cells floating into an extracellular mucin (Fig. 1).

Clinical Features

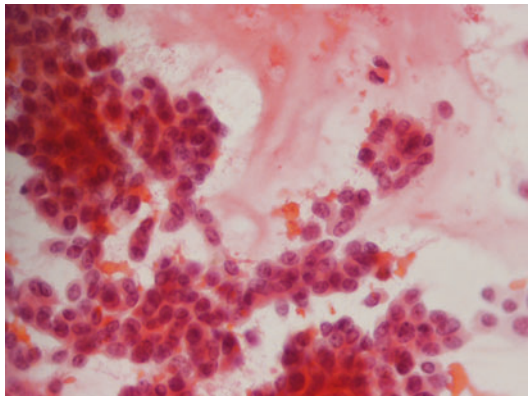
- **Incidence**
  The incidence varies from 0.8% to 5.3% of all breast cancers.
- **Age**
  Usually it affects at an older age than ordinary breast cancer with a median age of 62–68 y/o.
- **Sex**
  Rarely affects males.
- **Site**
  A specific and selective site is not reported.
- **Treatment/Outcome**
  The care of patients with breast cancer involves several disciplines including surgery, in general limited surgery with conservation of breast, sentinel node biopsy, limited radiation therapy, and appreciate adjuvant therapy with tailored targeted therapies. Pure mucinous carcinomas are associated with low rates of local and distant recurrence and excellent 5-year disease-free survival (Figs. 2 and 3).



Mucinous Breast Carcinoma, Cytological Findings, Fig. 2 Mucinous breast carcinoma. Abundant pools of mucus with scarce neoplastic cells (Papanicolaou stain)



Mucinous Breast Carcinoma, Cytological Findings, Fig. 4 Mucinous breast carcinoma. Observe signet-ring cells (Papanicolaou stain)



Mucinous Breast Carcinoma, Cytological Findings, Fig. 3 Mucinous breast carcinoma. Groups of neoplastic cells showing mild pleomorphism, characteristics of a low-grade carcinoma (H&E stain)

Macroscopy

This neoplasm tends to be a well-circumscribed nodule with pushing borders. The extracellular mucus is characterized by the evidence of pink gelatinous cut surface with hemorrhagic areas. The size ranges from 0.5 to 20 cm.

Microscopy

Cytologically, the specimen is defined by the presence of gelatinous material with variable cellularity. The key features are summarized by (1) abundant presence of mucin, (2) aggregates of uniform neoplastic cells, and (3) occasional evidence of signet-ring cells. The pure variant shows clusters of cells in a gelatinous extracellular mucinous material which is typically metachromatic in a Diff-Quick preparation. The cells show mild atypia identified in rare pleomorphism and irregularities with vesicular chromatin and little anisonucleosis.

Immunophenotype

The pure variant of mucinous carcinoma is ER and PR positive and HER2 non-amplified. MUC-1 and MUC-2 EMA and WT-1 expression are reported. In some cases also gross cystic disease fluid protein (GCDFP-15) is positive (Fig. 4).

Molecular Features

Transcriptomic and immunohistochemical studies have demonstrated that mucinous carcinomas are of luminal A molecular subtype. These tumors are divided in mucinous A and mucinous B, the latter showing a pattern of gene expression similar to

neuroendocrine tumors. Lacroix-Triki et al. found a relatively low level of genomic instability, including less frequent gains of 1q and 16p and losses of 16q and 22q, as compared with grade-matched estrogen receptor-positive invasive ductal carcinomas revealing a relative homogeneity of the molecular profiles of mucinous carcinomas.

Differential Diagnosis

Mucinous lesions constitute a wide spectrum of lesions including extracellular mucin associated with fibrocystic changes, mucocele, and mucinous carcinoma. Sometimes a differential diagnosis might be difficult. Mucocele-like lesions contain mucin with cluster of regular ductal cells; fibroadenoma may present some myxoid change in the stroma misdiagnosed as mucin. In the latter case, the age of patients (usually younger for fibroadenomas) and the typical fingerlike branching pattern of cells with naked bipolar nuclei in the background are helpful in achieving the correct diagnosis.

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Mucinous Ovarian Tumors, Cytological Findings

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Synonyms

Benign group: Adenoma and adenofibroma pseudomucinosum; Blastoma pseudomucinosum; Colloid cyst; Cystadenoma proliferans glandulare; Cystadenoma pseudomucinosum; Cystoid; Endocervicoma; Enteric ovarian adenoma; Gelatinous cystadenoma; Myxoid cyst; Ovarian pseudocolloid; Ovarian pseudomyxoma; Pseudomucinous adenoma; Pseudomucinous cystadenoma.

Borderline group: Atypical proliferative mucinous tumor of ovary, mucinous tumor of low malignant potential

Malignant group: Adenocarcinoma; Cancerized pseudomucinous blastoma, carcinoma adenopapillare; Carcinomatous adenoma; Enteroid ovarian carcinoma; Papillary pseudomucinous cystadenocarcinoma; Pseudomucinous adenocarcinoma; Pseudomucinous carcinoma, Pseudomucinous cystadenocarcinoma

Definition

Mucinous ovarian tumors are part of the surface epithelial-stromal tumor group of ovarian neoplasms, characterized by the presence of epithelial cells, mostly containing intracytoplasmic mucin. The epithelial cells may resemble that of the endocervix, intestine, or both. According to World Health Organization (WHO) classification, they are categorized as benign tumors (cystadenoma, adenofibroma, cystadenofibroma) borderline tumors (intestinal type and endocervical-like type), malignant tumors (adenocarcinoma and

adenocarcinofibroma/malignant adenofibroma/), then mucinous cystic tumor with mural nodules and mucinous cystic tumor with pseudomyxoma peritonei.

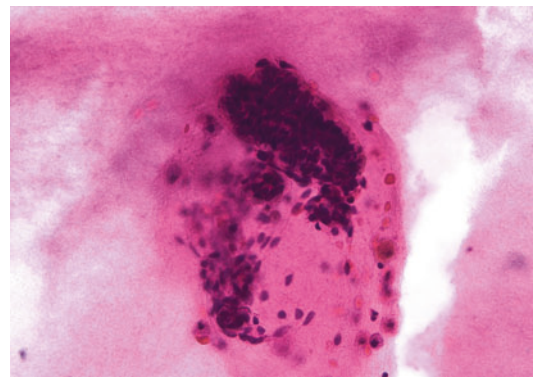
Microscopy

In diagnostics of ovarian tumors (non-neoplastic and neoplastic), a lot of different cytology samples are analyzed, before, during, as well as after operation: cervicovaginal smears, endocervical and endometrial samples, fine-needle aspiration (FNA) of ovaries, aspirate or washing of Douglas's space, washings and smears of peritoneum, imprints and scraps of tumors. Namely, cytologic analysis is important to differentiate non-neoplastic ovarian tumors from benign, borderline, and malignant tumors, then for clinical staging of disease, as well as in selecting the treatment and its assessment (Ivić 1976; Ganjei 1995). The cytologic interpretation of direct ovarian samples (FNA, imprints, scraps) could be difficult due to a variety of cystic and solid lesions, neoplastic and non-neoplastic. Therefore, it is very important to be familiar with the normal cytomorphologic features of ovaries, of the structures found during procedure (FNA), as well as of cytomorphology of tumor (ovarian and metastatic). The sample should be proper, cytologic preparation should be optimal, and a clinical history should be available prior to any cytologic interpretation. That means, for proper understanding and interpretation of cytologic fluid sample, it could be interpreted as unsuitable (only blood) or suitable (phagocytes). Suitable sample could be expressed as inadequate (only phagocytes are found as a sign of cystic lesion) or adequate (specific cells are found like granulosa cells, epithelium, mesothelial cells, etc.) (Ivić 1976; Ganjei 1995).

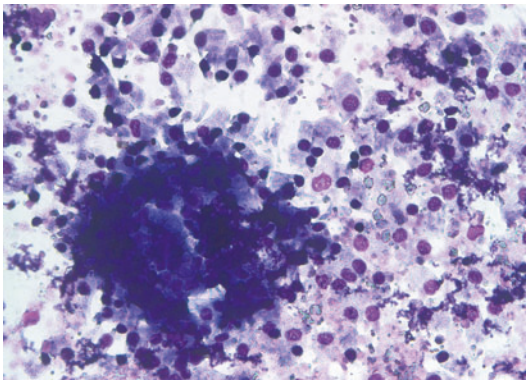
Fine-needle aspiration cytology in the preoperative investigation of ovarian tumors has been discouraged since puncture of a cystic carcinoma might cause intraperitoneal seeding (Trimbos and Hacker 1993), but intraoperative cytology enables a rapid diagnosis without fear of dissemination of ovarian cancer (Shahid et al. 2012).

Mucinous Ovarian Cystadenoma : Cytologic Findings

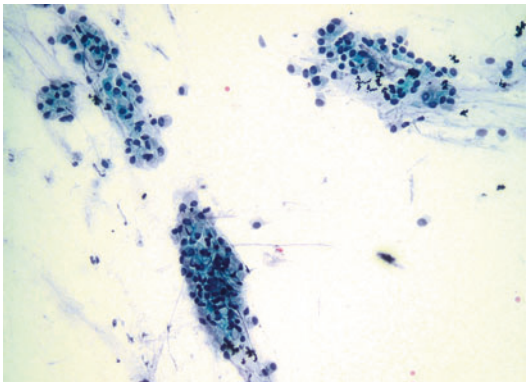
- **Fluid aspiration (FNA)**. *Macroscopically* fluid is viscous, gelatinous, mucoid, yellowish, sometimes blood tinged (Ivić 1976; Ganjei 1995; Kovačić et al. 1979; Kini 2011; Manek and Mahovlić 2010). *Microscopically* sample is often hypocellular, sometimes only macrophages, multinucleated giant cells, inflammatory cells and strings of mucin predominate at background. Clusters of mucinous cells without atypia are arranged in honeycomb or picket-fence (palisade) configurations (Fig. 1), presenting with usually eccentrically sited nuclei and abundant, finely vacuolated cytoplasm or a single large vacuole displaces the nucleus to the periphery. The nuclei are homogeneous and have finely granular evenly distributed chromatin. Single cells with vacuolization of cytoplasm could be very hard to differentiate between degenerating mucinous cells and true macrophages (Fig. 2) (Ivić 1976; Ganjei 1995; Kovačić et al. 1979; Kini 2011; Manek and Mahovlić 2010).
- **Imprint of mucinous cystadenoma**. Cellularity is usually high. Honeycomb sheets, palisading clusters, and single mucinous cells without atypia are present. Nuclei are eccentrically sited and cytoplasms are abundant and vacuolated, very often full of mucin. At background



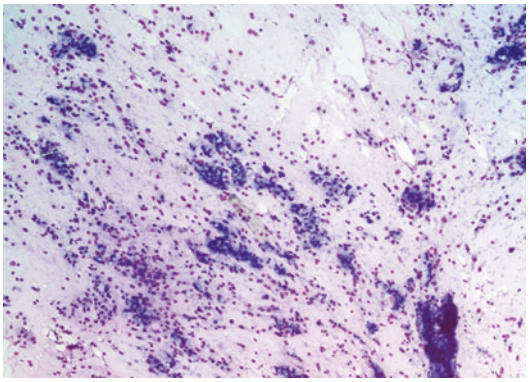
Mucinous Ovarian Tumors, Cytological Findings, Fig. 1 Cystadenoma mucinosum. A cluster of benign mucinous cells (honeycomb and picket-fence configuration) and strings of mucin at background (Fluid aspiration, Papanicolaou ×400)



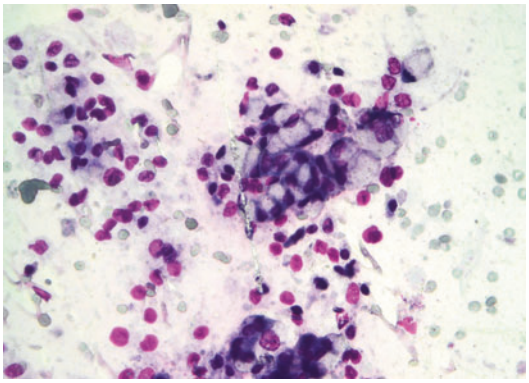
Mucinous Ovarian Tumors, Cytological Findings, Fig. 2 Cystadenoma mucinosum. A cluster and single benign mucinous cells (some hardly to differ from macrophages), dirty background (Fluid aspiration, MGG ×400)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 4 Cystadenoma mucinosum. A lot of benign mucinous cells in honeycomb and palisading configurations, mucin in background (Imprint, Papanicolaou ×200)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 3 Cystadenoma mucinosum. A lot of benign mucinous cells in honeycomb and palisading configurations, mucin in background (Imprint, MGG ×100)



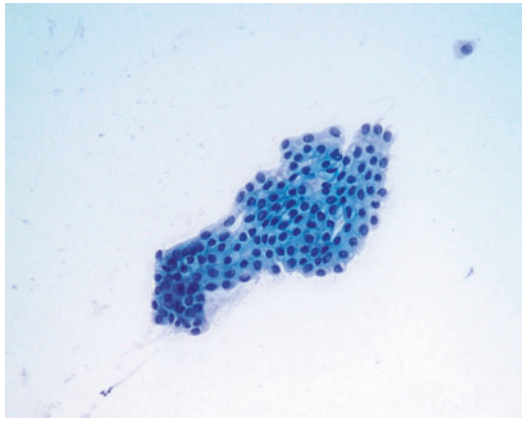
Mucinous Ovarian Tumors, Cytological Findings, Fig. 5 Cystadenoma mucinosum. Benign mucinous cells resembling goblet cells (Imprint, MGG ×400)

mucin is often present as well as stromal cells and naked nuclei of stromal cells (Figs. 3 and 4). Mucinous cells resemble either goblets cells or endocervical cells (Figs. 5 and 6) (Ivić 1976; Shahid et al. 2012; Audy-Jurković 1986).

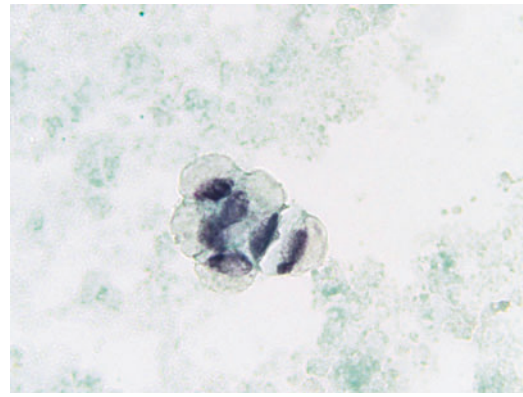
- **Peritoneal washing**. Usually mesothelial cells are found.

Mucinous Borderline Ovarian Tumors: Cytologic Findings

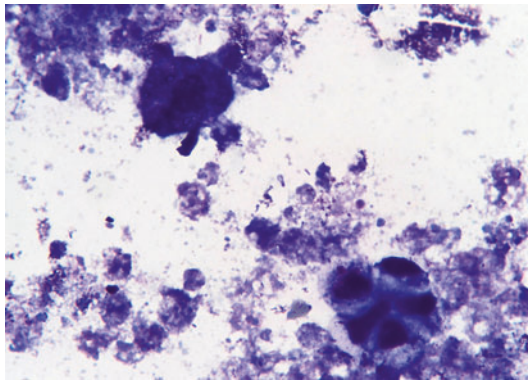
- **Fluid aspiration (FNA)**. *Macroscopically* fluid is gelatinous mucoid, thick, often blood tinged (Ivić 1976; Ganjei 1995; Kovačić et al. 1979; Kini 2011; Manek and Mahovlić 2010). *Microscopically* sample is more or less of greater cellularity with cytological features of atypia. Cells are more frequently in sheet-like groups or papillary configurations. Mucinous cells perform usually slightly atypical nuclei which may contain nucleoli, and cytoplasms with vacuoles of different sizes. The background contains a large number of histiocytes/phagocytes and mucin (Figs. 7 and 8) (Ivić 1976; Ganjei 1995; Kovačić et al. 1979; Kini 2011; Manek and Mahovlić 2010).
- **Imprint of mucinous borderline ovarian tumors**. Cellularity is variable, usually higher



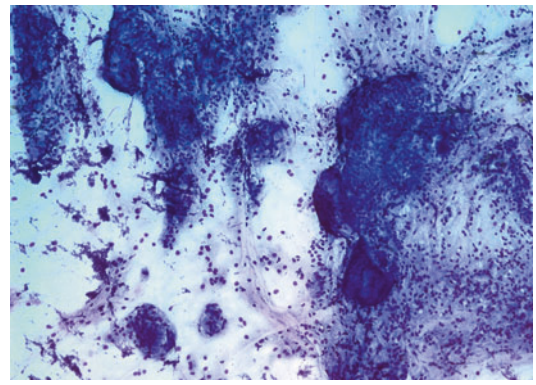
Mucinous Ovarian Tumors, Cytological Findings, Fig. 6 Cystadenoma mucinosum. Benign mucinous cells resembling endocervical cells (Imprint, Papanicolaou ×200)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 8 Mucinous borderline ovarian tumor. Small papillary configuration of slightly atypical mucinous cells with mild anisocytosis, phagocytes, dirty background (Fluid aspiration, Papanicolaou ×1,000)

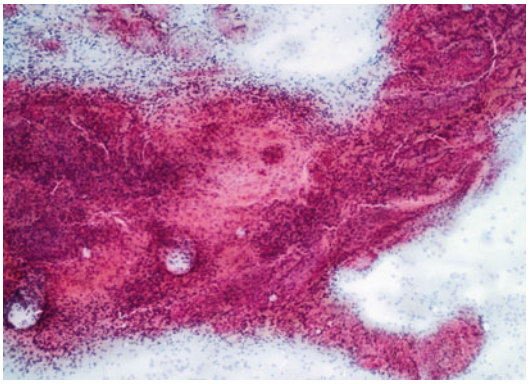


Mucinous Ovarian Tumors, Cytological Findings, Fig. 7 Mucinous borderline ovarian tumor. Small papillary configuration of slightly atypical mucinous cells with mild anisocytosis, phagocytes, dirty background (Fluid aspiration, MGG ×1,000)

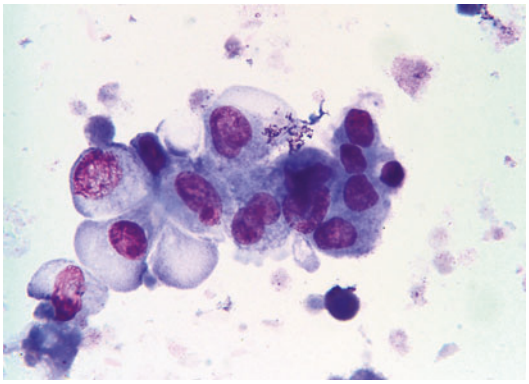


Mucinous Ovarian Tumors, Cytological Findings, Fig. 9 Mucinous borderline ovarian tumor. Abundant cellularity, mucin in background. Sheets, clusters, and papillary-like formations of mucinous cells with slight atypia and stratification (Imprint, MGG, ×100)

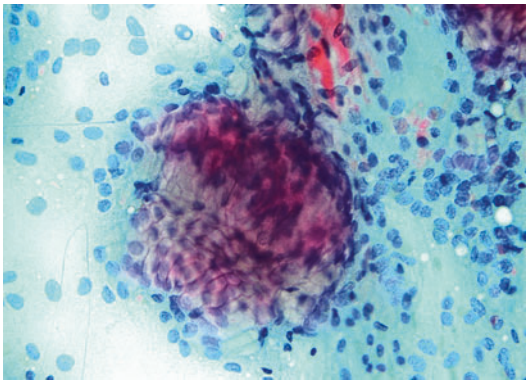
than in benign cyst (adenoma), i.e., moderate to abundant. Cells have higher N/C ratio than in benign counterpart, and are arranged in sheets, clusters, papillary formations characterized by mild anisocytosis and anisonucleosis, rare mitosis, sometimes with pronounced stratification and nucleoli (Figs. 9–11). Cells without atypia arranged in sheets and palisading clusters as well as single mucinous cells are very often present, too. Stromal cells can be seen in variable amount. The background is stream mucoid, often with a larger number of histiocytes/phagocytes (Ivić 1976; Audy-Jurković 1986). In endocervical-like mucinous borderline tumor, cells with abundant vacuolated cytoplasm are frequently found. Some of the cells have signet-ring-like configuration, mitosis are rare, and in background neutrophils and histiocytes admixed with epithelium are found. In intestinal type of mucinous borderline ovarian tumor, goblet cells are found. Nuclear atypia is low to moderate, mitosis are rare (Jiménez-Ayala and Jiménez-Ayala Portillo 2011).



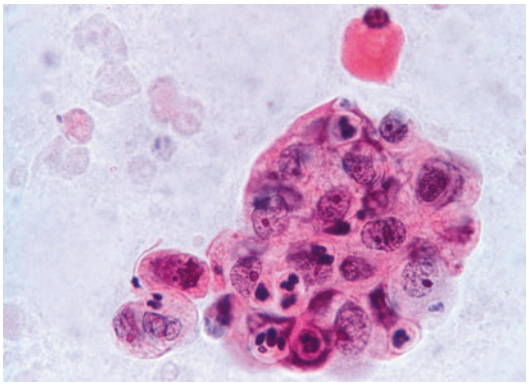
Mucinous Ovarian Tumors, Cytological Findings, Fig. 10 Mucinous borderline ovarian tumor. Abundant cellularity. Large branching sheets, clusters, and papillary-like formations of mucinous cells with mild atypia (Imprint, Papanicolaou, ×100)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 12 Mucinous ovarian adenocarcinoma. Malignant mucinous cells are pleomorphic in size, with anisonucleosis, irregular chromatin, and pronounced nucleoli (Fluid aspiration, MGG, ×1,000)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 11 Mucinous borderline ovarian tumor. Honeycomb and palisade-like configuration of mucinous cells with mild atypia and pseudostratification (Imprint, Papanicolaou, ×400)

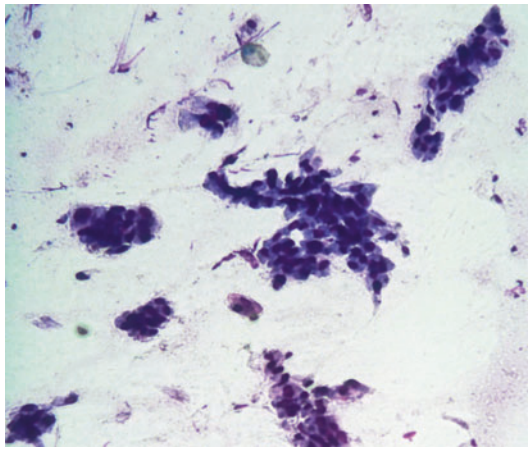


Mucinous Ovarian Tumors, Cytological Findings, Fig. 13 Mucinous ovarian adenocarcinoma. Malignant mucinous cells are pleomorphic, with anisonucleosis, pronounced nucleoli, and phagocytosis (Fluid aspiration, Papanicolaou, ×1,000)

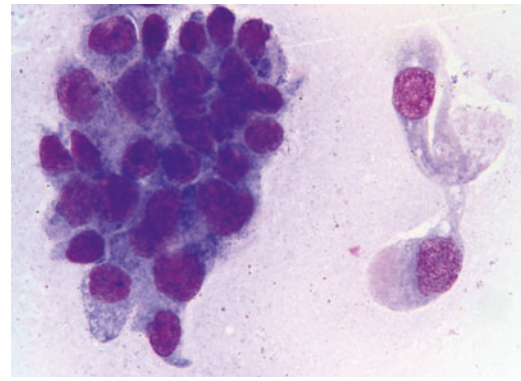
- **Peritoneal washing**. Presence of epithelial cells depends on progression of disease (peritoneal implants). Morphologically, cells are similar to those in cyst aspirate or imprint, but often more degenerated.

Mucinous Ovarian Adenocarcinoma

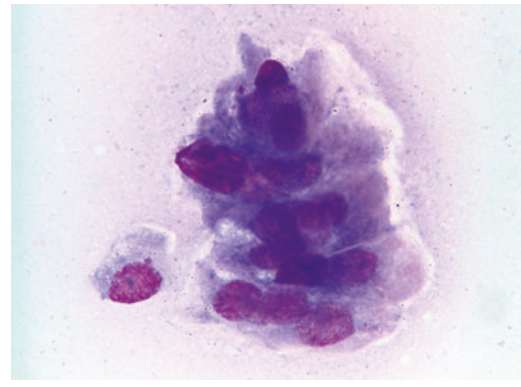
- **Fluid aspiration (FNA)**. *Macroscopically* fluid is usually thick, stringy, gelatinous, blood tinged (Ivić 1976; Ganjei 1995; Kovačić et al. 1979; Kini 2011; Manek and Mahovlić 2010). *Microscopically* sample is of variable cellularity, often in the background thick mucin, histiocytes/phagocytes, and necrosis are found. Cells have high N/C ratio, and are arranged in loosely cohesive groups, syncytial tissue fragments with papillary configuration, with or without branching. Cells are pleomorphic in size, nuclear membrane usually irregular, chromatin with parachromatin clearing and pronounced nucleoli, while cytoplasms are abundant with single or multiple vacuoles (Figs. 12 and 13). In the case of poor differentiated mucinous adenocarcinomas, it is difficult



Mucinous Ovarian Tumors, Cytological Findings, Fig. 14 Mucinous ovarian adenocarcinoma. Loosely cohesive groups of malignant mucinous cells (Imprint, MGG ×200)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 15 Mucinous ovarian adenocarcinoma. A loosely cohesive cluster of malignant mucinous cells, polarity is disturbed, anisonucleosis, pronounced nucleoli, and abundant cytoplasms (Imprint, MGG ×1,000)

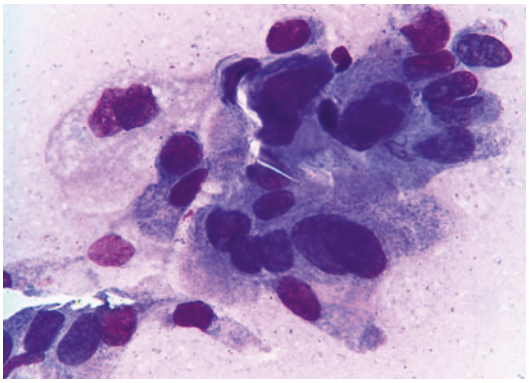


Mucinous Ovarian Tumors, Cytological Findings, Fig. 16 Mucinous ovarian adenocarcinoma. Malignant mucinous cells in multilayered palisading and with abundant cytoplasms (Imprint, MGG ×1,000)

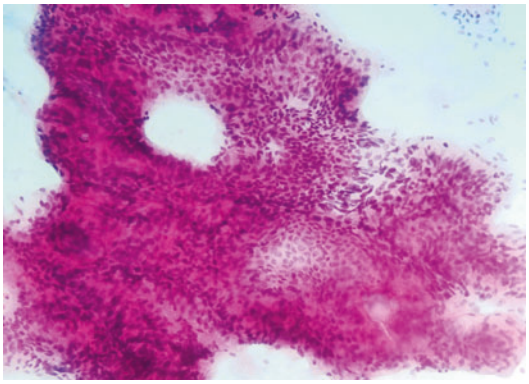
to differentiate them from other types of adenocarcinomas (Manek and Mahovlić 2010; Jiménez-Ayala and Jiménez-Ayala Portillo 2011).

- **Imprint of mucinous ovarian adenocarcinoma**. Cellularity is variable, usually high. There is usually abundant mucus as well as necrosis in the background. Among mucin, malignant cells are arranged in loosely cohesive groups, and syncytial tissue fragments without and/or with an acinar or papillary pattern, or multilayered palisades, polarity is disturbed. The malignant cells are large, usually with variable, vacuolated cytoplasm. Anisocytosis and anisonucleosis are pronounced, sometimes multinucleation is seen (Figs. 14–19) (Ivić 1976; Shahid et al. 2012; Audy-Jurković 1986).
- **Peritoneal washing and ascites**. Presence of malignant epithelial cells depends on progression of disease. Morphologically, malignant cells are similar to those in ovary, ranging from small to large, occurring as individually dispersed cells, exhibiting a markedly pleomorphic pattern or desquamating in papillary pattern and acinar formation. Mucin production is characterized by large distended cytoplasmic vacuoles. The background is often hemorrhagic, dirty, and necrotic (Figs. 20 and 21).

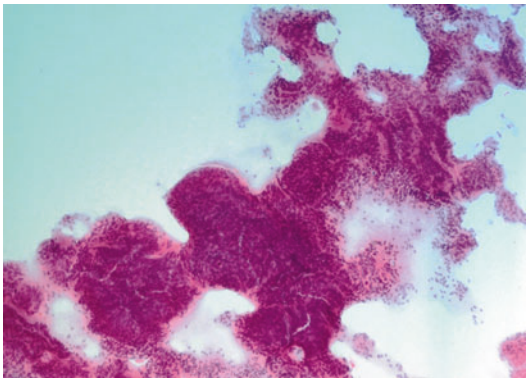
Rupture of the ovarian mucinous cystadenoma or cystadenocarcinoma may cause **pseudomyxoma peritonei**. It is characterized by a large abdominal collection of gelatinous or mucinous material as a result of the implantation of the peritoneum with neoplastic cells, primarily of the ovary and appendix (Manek and Mahovlić 2010). In cytologic specimens (Koss 2006a), the mucinous background is the hallmark, characterized by thick



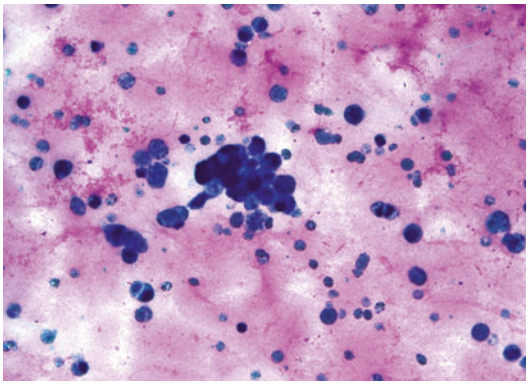
Mucinous Ovarian Tumors, Cytological Findings, Fig. 17 Mucinous ovarian adenocarcinoma. Malignant mucinous cells in loosely cohesive cluster with disturbed polarity, partially palisading with abundant cytoplasms (Imprint, MGG ×1,000)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 19 Mucinous ovarian adenocarcinoma. Cluster of crowded malignant mucinous cells, showing open glandular pattern (Imprint, Papanicolaou × 200)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 18 Mucinous ovarian adenocarcinoma. Branching sheets and clusters of malignant mucinous cells, somewhere showing open glandular pattern (Imprint, Papanicolaou ×100)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 20 Mucinous ovarian adenocarcinoma. Papillary configuration of malignant mucinous cells, dirty background with mucin (Peritoneal washing, MGG ×400)

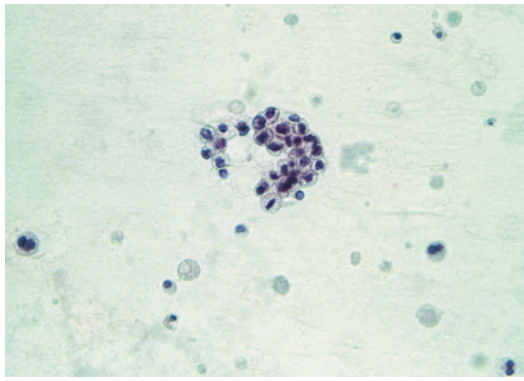
strands of mucinous material, easily recognized by routine staining (Papanicolaou, MGG), that contains a dual population of mesothelial and fibroblastic cells. Epithelial cells (benign or malignant) may be admixed (Fig. 22).

Immunophenotype

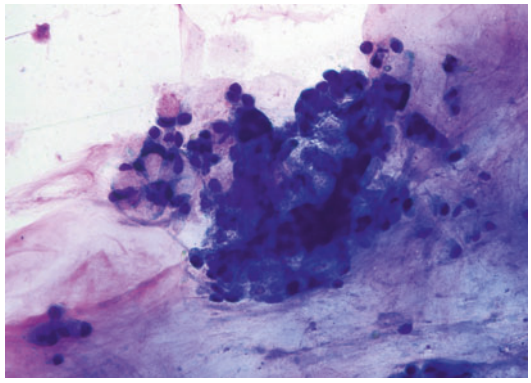
The cells of mucinous ovarian cystadenocarcinoma react positively to CK7 (Fig. 23) CK20 (Fig. 24), CEA (Fig. 25), and CDX2 and negatively to CA125 (Kini 2011).

Differential Diagnosis

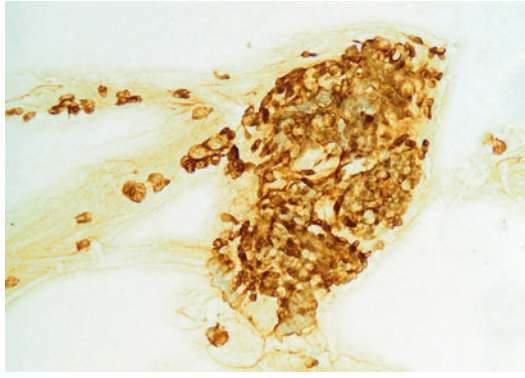
It includes metastatic mucinous adenocarcinoma originating mostly in gastrointestinal tract (large intestine, appendix, stomach, pancreas, biliary tract), cervix and endometrioid carcinoma with mucinous differentiation, than Sertoli-Leydig tumors with heterologous mucinous component, as well as mucinous borderline tumors. Diagnosis of a metastatic origin includes mostly bilaterality, multinodular growth, superficial and



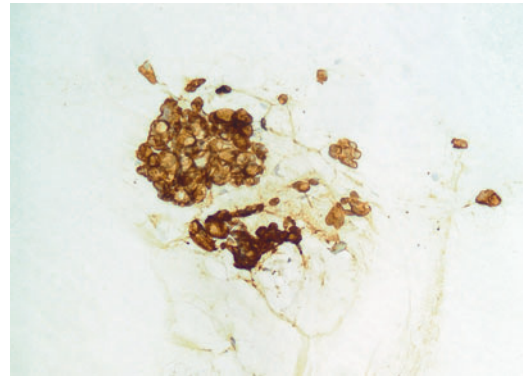
Mucinous Ovarian Tumors, Cytological Findings, Fig. 21 Mucinous ovarian adenocarcinoma. Malignant mucinous cells showing disturbed polarity, anisocytosis, and honeycomb pattern (Peritoneal washing, Papanicolaou ×400)



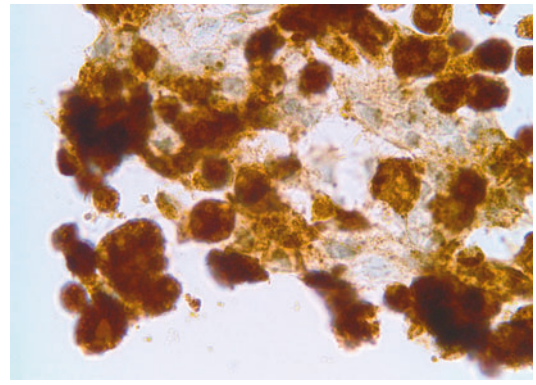
Mucinous Ovarian Tumors, Cytological Findings, Fig. 22 Pseudomyxoma peritonei Cluster of malignant mucinous cells (Imprint MGG ×400)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 23 Mucinous ovarian adenocarcinoma. Immunocytochemistry. Positive CK7 in malignant mucinous cells (Imprint, Envision^{TM} Flex, High pH ×200)

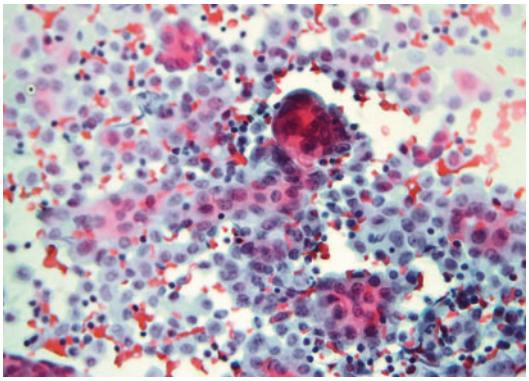


Mucinous Ovarian Tumors, Cytological Findings, Fig. 24 Mucinous ovarian adenocarcinoma. Immunocytochemistry. Positive CK20 in malignant mucinous cells (Imprint, Envision^{TM} Flex, High pH ×200)

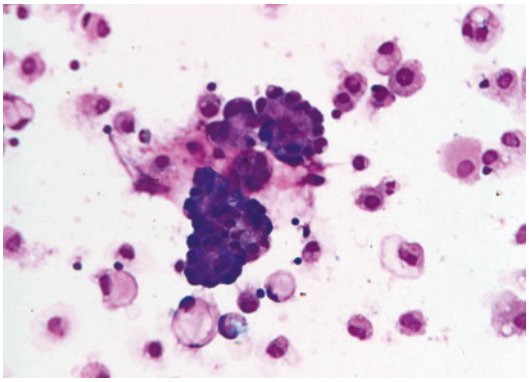


Mucinous Ovarian Tumors, Cytological Findings, Fig. 25 Mucinous ovarian adenocarcinoma. Positive CEA in malignant mucinous cells (Peritoneal washing, Envision^{TM} Flex, High pH ×1000)

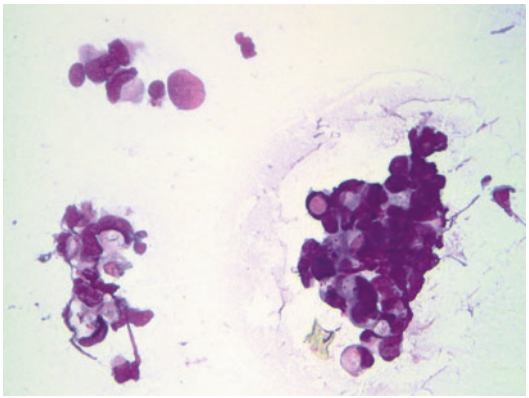
extraovarian dissemination (Jiménez-Ayala and Jiménez-Ayala Portillo 2011). **Krukenberg tumor** is well-known manifestation of metastatic gastrointestinal cancer to the ovary. Cytologically, malignant cells are presented as signet-ring cell type (Figs. 26–29), which are almost spherical, with a large hyperchromatic nucleus pushed to the periphery by a large cytoplasmic mucous vacuole (Koss 2006b).



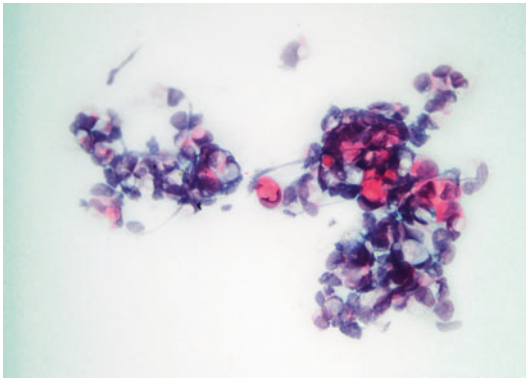
Mucinous Ovarian Tumors, Cytological Findings, Fig. 26 Krukenberg tumor. Papillary configuration of malignant signet-ring type cells (Ascites, Papanicolaou ×400)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 27 Krukenberg tumor. Papillary configuration of malignant signet-ring type cells (Ascites, MGG ×400)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 28 Krukenberg tumor. Clusters of malignant signet-ring type cells (Imprint, MGG ×400)



Mucinous Ovarian Tumors, Cytological Findings, Fig. 29 Krukenberg tumor. Clusters of malignant signet-ring type cells (Imprint, MGG ×400)

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Mucoepidermoid Carcinoma, Cytological Findings

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Synonyms

Mucoepidermoid tumor (inappropriate)

Definition

Mucoepidermoid carcinoma (MEC) is a primary salivary adenocarcinoma showing biphasic, squamous, and mucus-secreting differentiation (Barnes et al. 2005; Ellis and Auclair 2008). Depending on differentiation, MEC may be high- or low-grade malignancy. MEC, together with adenoid cystic carcinoma and acinic cell carcinoma, belongs to the group of the most common salivary malignant entities.

Clinical Features

- **Incidence**
  It is well recognized that MEC is the most common malignant primary salivary tumor representing approximately 30% of all malignancies and 16% of all salivary gland lesions.
- **Age**
  There is a harmonious distribution ranging from the second to eighth decades of life with a pic incidence in the sixth pediatric cases known, MEC being the most common malignancy in patients younger than 20 years.
- **Sex**
  There is a slight female predilection.
- **Site**
  MECs arise in major and minor salivary glands in equal percentage, parotid gland and palate being the most common sites for major and minor salivary glands, respectively. Intraosseous (mandible, maxilla) primary localizations are also known.
- **Treatment**
  Surgery is usually an initial treatment.
- **Outcome**
  High-grade carcinomas may recur and have metastatic evolution. Metastatic evolution from low-grade tumors is exceptional.

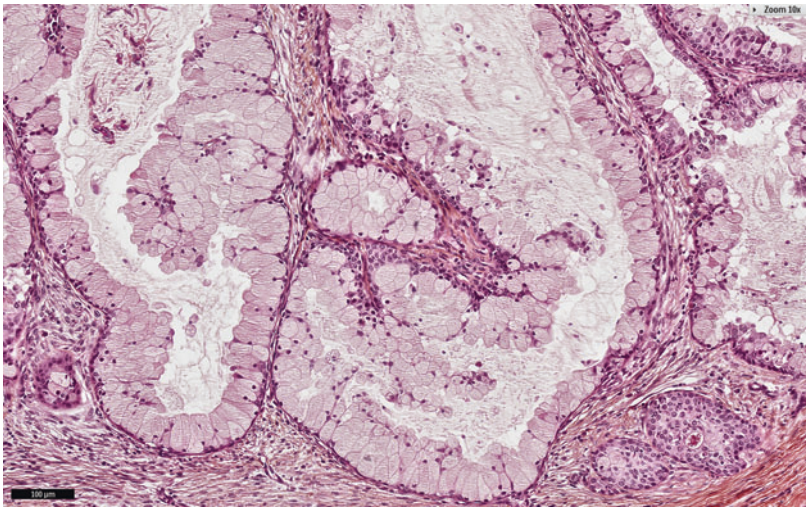
Macroscopy

Some low-grade tumors may simulate unilocular, well-encapsulated cyst. High-grade MECs are usually gray-white, poorly circumscribed masses with cystic or necrotic spaces (Fig. 1).

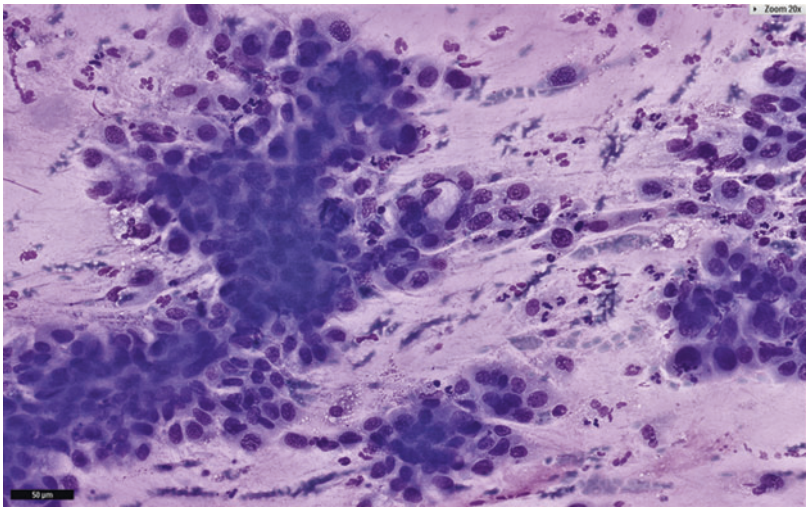
Microscopy

Smears in MEC are usually hypercellular and cell rich and stroma rich. MEC belongs to the group of tumors exhibiting predominant epithelial cell morphology.

Despite of the low- or high-grade differentiation, smears show three classical components: intermediate cells, squamous/squamoid cells, and mucus-secreting cells (Klijanienko and Vielh 1997a). Intermediate cells are pathognomic for MEC. They are middle sized and have round nuclei with mild or moderate atypia and prominent nuclei. These cells are usually cohesive in small clusters. Cytoplasm is moderately abundant, clarified, and eosinophilic. Intranuclear inclusions may be seen. Squamous/squamoid cells are less frequent in low-grade tumors, but usually present in high-grade tumors (Fig. 2). Cytoplasm may be gray-blue with marked keratinization. Mucus-secreting cells are constantly



Mucoepidermoid Carcinoma, Cytological Findings, Fig. 1 Mucoepidermoid carcinoma, low grade. Intermediate cells and mucus-secreting cells in pseudopapillary clusters and cyst formation (H&E stain)

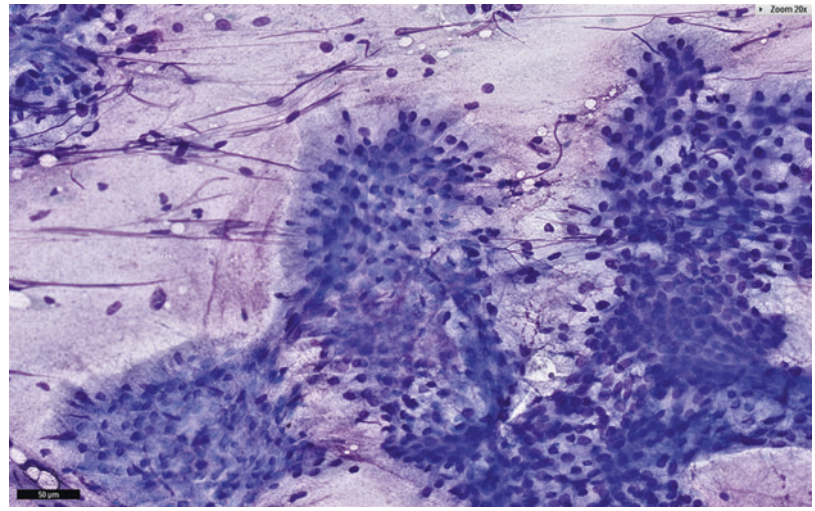


Mucoepidermoid Carcinoma, Cytological Findings, Fig. 2 Mucoepidermoid carcinoma, high grade. Intermediate and squamoid malignant cells. The background is mucoid and mucus-secreting cells are well identified as clear areas within the clusters (MGG stain)

seen in low-grade tumors and rare in high-grade tumors (Fig. 3). They present a large morphologic variability: some cases have a goblet-like appearance, whereas others frequently have a pseudohistiocytic morphology. Mucus-secreting pseudohistiocytic cells exhibit large, multivacuolated cytoplasm and central small nuclei. Occasionally, clear cells and oncocytic-like cells are found. Moreover, rare smears may show mast cells associated with oncocytic-like cells. The background is rich or scant in mucin in low-grade or high-grade tumors, respectively. Inflammatory infiltrates may be also observed in low-grade tumors.

Immunophenotype

Immunostaining does not play an important role in the diagnosis. Epithelial cells are immunoreactive for cytokeratins and EMA. S-100 protein is usually not expressed as opposed to p63 which is almost always present in MEC, an immunoprofile



Mucoepidermoid Carcinoma, Cytological Findings, Fig. 3 Mucoepidermoid carcinoma, low grade. Intermediate cells and mucus-secreting cells in pseudopapillary clusters. The background is cystic. Compare with Fig. 1 (MGG stain)

similar to that of hyalinizing clear cell carcinoma (Milchgrub et al. 1994).

Molecular and Cytogenetic Features

Allelic loss of 2q, 5p, 12p, and 16q has also been demonstrated in some MEC. However, despite its structural and cellular heterogeneity, MEC is uniquely characterized by specific translocations. The first t(11;19)(q21;p13) translocation results in a fusion between the *MECT1* and the *MAML2* genes, in more than 50% of cases disrupting the Notch signaling pathway (Weinreb 2013). Fusion-positive cases showed significantly better survival than fusion-negative cases, suggesting that *MECT1-MAML2* may represent a potentially prognostic molecular marker in MEC. The second translocation t(11;15)(q21;q26) results in a *CRTC3-MAML2* fusion found in about 6% of tumors is also specific but does not seem of prognostic value. In high-grade tumors, HER2 or EGFR gene abnormality could play an important role as well as in the progression from *MAML2* fusion-positive low-/intermediate-grade to high-grade in a subset of MEC (Bell and El-Naggar 2013; Nakano et al. 2013; Clauditz et al. 2012).

Differential Diagnosis

High-grade MEC carcinoma should be differentiated from squamous cell carcinoma (SCC), salivary duct carcinoma (SDC), and MEC ex pleomorphic adenoma (PA). High-grade MEC may mimic SCC because of its paucity in mucus-secreting cells. Intermediate cells and three-dimensional clusters of cells among minimally keratinizing malignant cells and rare keratin debris are very helpful features of MEC when mucus-secreting cells are absent (Klijanienko and Vielh 1998a; Lussier et al. 2000).

When smears are composed of atypical intermediate cells, the differential diagnosis with SDC should be considered. SDC has typical adenocarcinomatous morphology with oncocytic-like cells and comedonecrosis (Klijanienko and Vielh 1998b).

When the smears show abundant mucinous background and scattered oncocytic-like cells, the differential diagnosis of MEC with WT should be made. Typical WTs are composed of oncocytic cells with mast cells within a large lymphocytic background. Usually WTs lack cytonuclear atypia and background is rather pseudonecrotic than necrotic (Klijanienko and Vielh 1997b). When specific patterns of PA are intermingled with

MEC, a diagnosis of carcinoma ex PA should be done. The differential diagnosis may also be guided by a clinical history of PA (Klijanienko et al. 1998).

Low-grade MEC should be differentiated from salivary cyst (SC), pleomorphic adenoma (PA) with background mucoid, and Küttner tumor (KT, chronic sclerosing sialadenitis). The differential diagnosis is not possible when the smears only contain mucoid stroma with no epithelial cells. However, some mucus-secreting cells may mimic histiocytes from SC. Special caution should be made when aspirated liquid is mucoid or dense, keeping in mind that the nonrecognition between low-grade MEC and SC is a source of a large percentage of false-negative results reported in the cytological literature. Some PA may contain mucoid background. The presences of chondromyxoid stroma and plasmacytoid myoepithelial cells are pathognomic for PA (Klijanienko and Vielh 1996). Chronic sialadenitis (Küttner tumor) may also mimic low-grade MEC, because of dark ductal cells resembling intermediate cells, background mucus, and inflammatory cells. For these reasons, we suggest that all submandibular gland tumors be underdiagnosed when a MEC is suspected, with referral of the patient for intraoperative frozen sections examination.

The absence of *MAML2* rearrangement and/or positivity of *AWSR1-ATF* fusion by FISH is useful to make the diagnosis of mucinous differentiation in hyalinizing clear cell carcinoma as it is characteristic of this entity (Weinreb 2013).

CRTC1-MAML2 or *CRTC3-MAML2* are absent in metaplastic Warthin tumor and metaplastic pleomorphic adenoma of salivary glands (Skálová et al. 2013).

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Myxoid Sarcomas, Cytological Findings

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Synonyms

Extraskeletal myxoid chondrosarcoma; Low-grade fibromyxoid sarcoma; Myxoid liposarcoma; Myxoid malignant fibrous histiocytoma(myxoid variant)

Definition

Soft tissue sarcoma with production of a myxoid background matrix.

Clinical Features

- **Incidence**
  Myxoid liposarcoma: 3 per 1000 000. Low-grade fibro myxoid sarcoma: rare. Extraskeletal myxoid chondrosarcoma: 1per 1 000 000. Malignant fibrous histiocytoma (myxoid variant): relatively common.
- **Age**
  Adults and elderly patients.
- **Sex**
  No sex predilection.
- **Site**
  Myxoid liposarcoma: deep soft tissue, mostly thigh. Low-grade fibromyxoid sarcoma: trunk, proximal extremities. Extraskeletal myxoid chondrosarcoma: deep soft tissue of proximal extremities. Malignant fibrous histiocytoma (myxoid variant): limbs.
- **Treatment**
  Surgery, chemotherapy, radiation.
- **Outcome**
  Low-grade variants have high survival rate if local excision is radical. High-grade tumors have often metastases at the time of operation.

Microscopy

Myxoid liposarcoma: rich myxoid material with delicate capillaries, round tumor cells with poorly defined cytoplasm, lipoblasts.

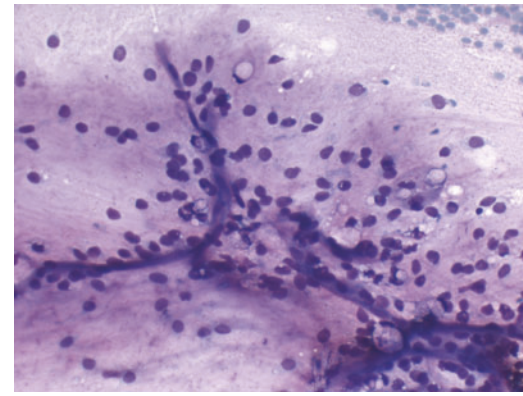
Low-grade fibromyxoid sarcoma: myxoid background, sometimes collagen, vessels, spindle cells with slight atypia.

Extraskeletal myxoid chondrosarcoma: myxoid-chondromyxoid background, round to oval tumor cells with sparse distinct cytoplasm, cords, or clusters of tumor cells. Anaplastic cells in rare cases.

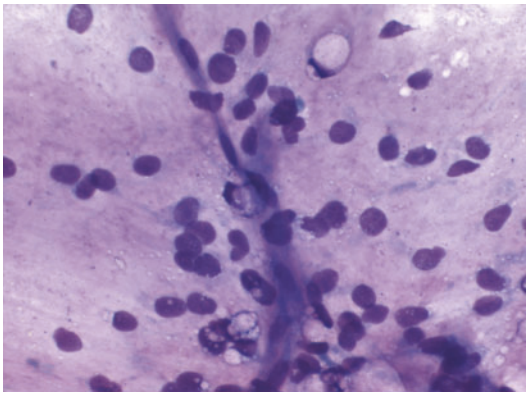
Malignant fibrous histiocytoma (myxoid variant): myxoid often bloody background, spindle cells with moderate to pronounced atypia, vessel fragments, and necrosis (Figs. 1–4).

Immunophenotype

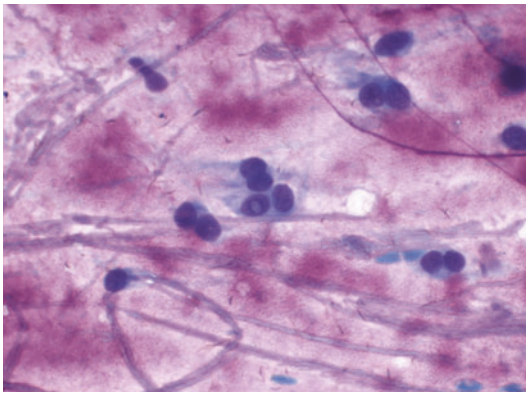
Myxoid liposarcoma: vimentin, S100.



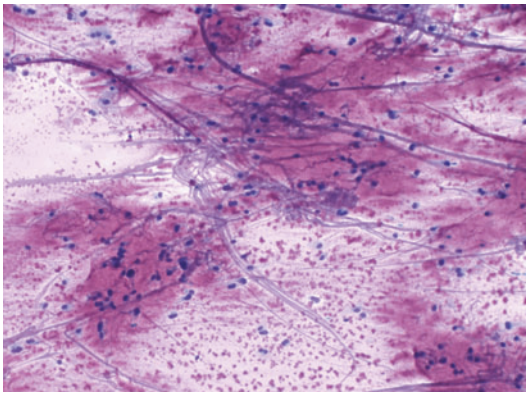
Myxoid Sarcomas, Cytological Findings, Fig. 1 Myxoid liposarcoma: tissue fragment with branching capillaries and monomorphic tumor cells in a myxoid matrix (Low magnification, MGG)



Myxoid Sarcomas, Cytological Findings, Fig. 2 Myxoid liposarcoma: a thin capillary with loosely attached monomorphic round to oval cells with sparse cytoplasm. One multinucleated lipoblast (High magnification, MGG)



Myxoid Sarcomas, Cytological Findings, Fig. 4 Low-grade fibromyxoid sarcoma: monomorphic tumor cells with round nuclei and poorly defined elongated cytoplasm (High magnification, MGG)



Myxoid Sarcomas, Cytological Findings, Fig. 3 Low-grade fibromyxoid sarcoma: loose myxoid fragments with few spindle tumor cells. *Rope-shaped* collagen fibers (low magnification, MGG)

Low-grade fibromyxoid sarcoma: vimentin.
Extraskeletal myxoid chondrosarcoma: vimentin.
Malignant fibrous histiocytoma (myxoid variant): vimentin, sometimes actin.

Molecular Features

Myxoid liposarcoma: t(12;16).
Low-grade fibromyxoid sarcoma: no specific changes.
Extraskeletal myxoid chondrosarcoma: t(9;22) or t(9;17).
Malignant fibrous histiocytoma (myxoid variant): no specific aberrations.

Differential Diagnosis

Benign myxoid tumors such as myxoma, ossifying myxoid tumor, myxoid lipoma, myxoid neurofibroma, and nodular fasciitis.

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N

Negative for Intraepithelial Lesion, Cytological Findings

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Synonyms

Negative; Normal

Definition

Cervical cytology specimens which do not contain abnormal epithelial cells are reported as "negative for intraepithelial lesion" under the Bethesda System terminology (Solomon et al. 2002). Alternative classification systems, such as those used by the World Health Organisation and the National Health Service Cervical Screening Programme in the UK, prefer the terms "normal" and "negative," respectively. Various optional comments may be added to the report but are not considered essential if they do not affect patient management. Examples include the cellular changes associated with inflammation, radiation, intrauterine contraceptive device, and atrophy. The presence of certain organisms, such as *Trichomonas vaginalis, Candida*, and herpes virus–associated cells, may be reported alongside the "negative for intraepithelial lesion" category as they have potential clinical relevance.

Clinical Features

- **Incidence**
  The proportion of cervical cytology reports issued as *negative for intraepithelial lesion* is population dependent and will also reflect interobserver variations in the interpretation of samples. In well-screened populations with a relatively low prevalence of cervical abnormalities, a large proportion of cervical cytology reports will be normal. A survey conducted by the College of American Pathologists in 2003 following the introduction of Bethesda 2001 terminology revealed that approximately 93% of cervical cytology specimens reported by 1751 laboratories were normal (Davey et al. 2004). In higher risk populations, such as women attending genitourinary medicine or colposcopy clinics, normality rates for cervical cytology can be expected to be lower.
- **Age**
  The majority of cervical cytology results across all screening ages are *negative for intraepithelial lesion*. As most, if not all, cervical intraepithelial lesions are related to infection with ▶ human papillomavirus (HPV). Pap test normality rates are generally lowest in populations in which the prevalence of HPV

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infection is highest, i.e., in relatively young sexually active women. This is confirmed in screening programs for which age-stratified screening data exist (The NHS Information Centre, Public Health Indicators and Population Statistics Team 2010).

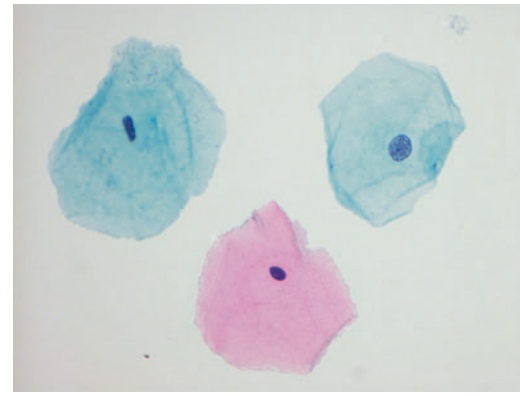
- **Sex**
  Relevant only to females.
- **Site**
  This terminology is used exclusively in the reporting of cervical and vaginal samples.
- **Treatment**
  Not relevant.
- **Outcome**
  The risk of developing cervical cancer in women with normal cervical cytology depends upon several factors, including screening frequency and age. Saseini et al estimate that 5-yearly screening offers considerable protection (83%) against cervical cancer at ages 55–69 years, but that 3-yearly screening is required in younger women to give the same protective effect (Sasieni et al. 2003). Data such as these suggest that regular negative cervical cytology is associated with a low risk of cervical cancer.

Macroscopy

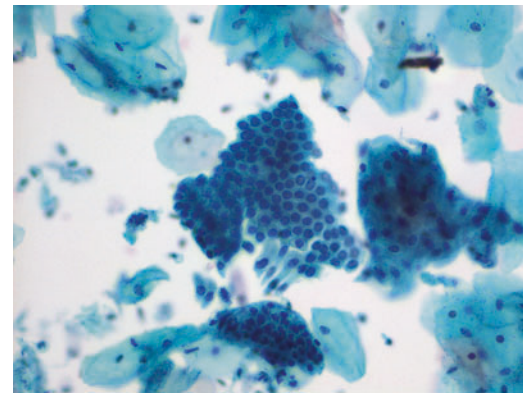
The normal cervix has a smooth, glistening mucosal surface with a pink coloration and a small, round-to-oval shaped os.

Microscopy

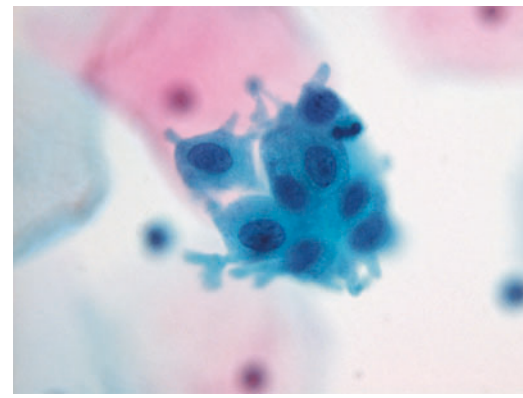
Cervical cytology preparations that are negative for intraepithelial lesion contain a variety of morphologically normal epithelial cells arising from the mucosa of the lower genital tract. Cell types may include squamous cells from stratified squamous epithelium of the ectocervix (Fig. 1), columnar cells from the endocervical canal (Fig. 2), squamous metaplastic cells from the transformation zone (Fig. 3), and possibly glandular cells from the endometrium (Fig. 4). Normal epithelial cells are characterized by nuclei with regular chromatin and smooth nuclear



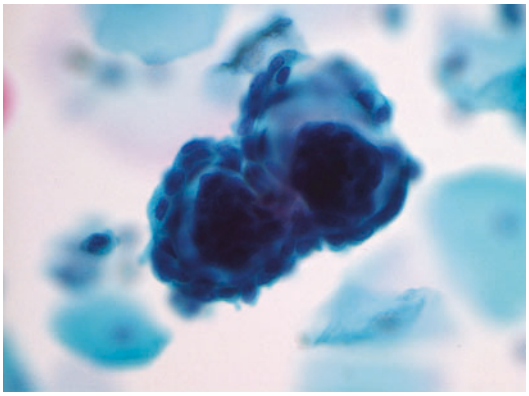
Negative for Intraepithelial Lesion, Cytological Findings, Fig. 1 Normal cervical squamous epithelial cells (Pap stain)



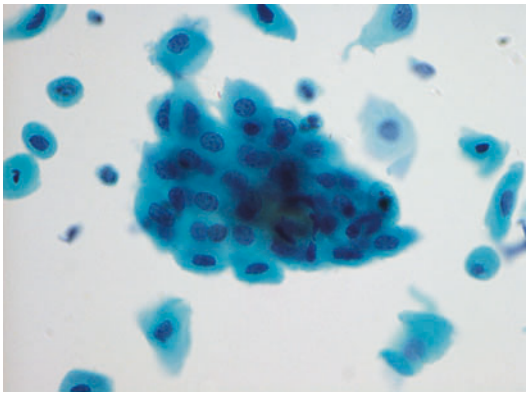
Negative for Intraepithelial Lesion, Cytological Findings, Fig. 2 Normal endocervical cells (Pap stain)



Negative for Intraepithelial Lesion, Cytological Findings, Fig. 3 Normal squamous metaplastic cells (Pap stain)



Negative for Intraepithelial Lesion, Cytological Findings, Fig. 4 Normal endometrial cells (Pap stain)



Negative for Intraepithelial Lesion, Cytological Findings, Fig. 5 Normal parabasal cells mimicking high-grade squamous intraepithelial lesion (Pap stain)

membranes. Nucleocytoplasmic ratios are cell-type dependent but are generally low to moderate.

Immunophenotype

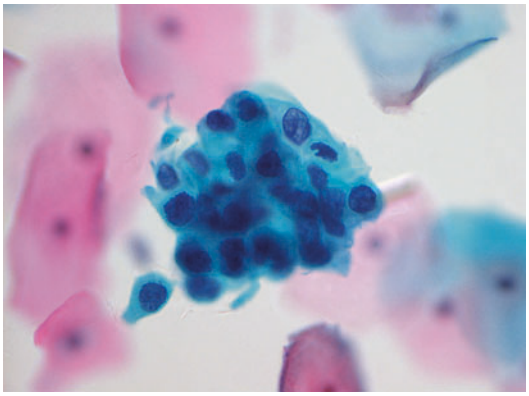
Embryologically, cervical epithelial cells are derived from the paramesonephric (mullerian) ducts. Protein expression profiling of normal cervical epithelial cells has been examined in numerous studies aiming to discover new biomarkers of neoplasia. Among the molecules expressed in normal cervical tissue are the cytokeratins, steroid hormone receptors, carcinoembryonic antigen (CEA), Ki-67, p53, S100, smooth muscle actin, and many more.

Molecular Features

Non-neoplastic cervical epithelial cells are characterized by a stable genomic structure without significant alterations in the genes associated with regulation of the cell cycle.

Differential Diagnosis

Any of the normal cervical epithelial cell types are a potential source of diagnostic error. Of particular importance are cells with a relatively high nuclear-to-cytoplasmic ratio, such as parabasal cells (Fig. 5), squamous metaplastic cells, endometrial cells, and endocervical cells. Such cells can mimic neoplastic cells almost to perfection, particularly, in the presence of inflammation, atrophy, or cell degeneration (Fig. 6).



Negative for Intraepithelial Lesion, Cytological Findings, Fig. 6 Cells from high-grade squamous intraepithelial lesion (Pap stain)

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Neuroblastoma, Cytological Findings

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Synonyms

Sympathicoblastoma

Definition

Neuroblastoma NOS is an embryonal tumor arising from cells of the developing sympathetic nervous system. The main cell of this tumor is the neuroblast (pluripotent sympathetic cell). The etiopathogeny of neuroblastomas is still an enigma. Familiar cases as well as a small percentage of sporadic cases have been associated with anaplastic lymphoma kinase (ALK) gene mutations.

Clinical Features

- **Incidence**
  Neuroblastoma accounts for about 8% of all childhood cancers and is the most common solid tumor of childhood (Van Noese and Versteeg 2004). Most tumors are sporadic; however in a small percentage of cases, 2%, a familial incidence is reported. Its association with Beckwith-Wiedemann Syndrome, Hirschsprung's disease, neurofibromatosis, or as a complication of fetal hydantoin syndrome has been described in a minority of cases.

  Symptoms can be due to local growth and local compression of anatomical structures (e.g., abdominal pain, neurologic symptoms due to invasion of spinal cord, or urinary tract, Horner syndrome, airway obstruction, etc.).

  Symptoms can also be due to metastatic disease (e.g., bone pain, blueberry muffin baby, periorbital ecchymosis).

  Finally, symptoms can be associated with paraneoplastic syndromes. There are mainly two paraneoplastic syndromes: diarrheal due to tumor secretion of vasoactive peptide secretion and opsoclonus and myoclonus, due to immunologic mechanisms.
- **Age**
  The age distribution is as follows: 40% of patients are younger than 1 year when diagnosed, 35% are aged from 1 to 2 years, and 25% are older than 2 years. After 10 years old, the disease is rare.
- **Sex**
  Males seem to have higher incidence and white children seem to be more affected than black children.
- **Site**
  In the developing embryo, neuroblasts migrate along the euroaxis to populate the sympathetic ganglia, the adrenal medulla as well as other sites all along the pathway followed by neuroblasts during the embryo development. The abdominal cavity is the more usual site of appearance (40% in the adrenal and 25% in the paraspinal ganglia), other sites, like the thoracic cavity, 15%, pelvis, 5%, or cervical location in 3% of the cases, are rarer.

  Curiously, distribution varies with age, and so thoracic and cervical tumors affect more frequently infants while older children are more prone to abdominal location.
- **Treatment**
  For treatment purposes, patients are classified into three Risk Groups: Low, Intermediate, and High Risk.

Treatment is as follows (Hsu et al. 2006; Maris et al. 2007; Ambros and Ambros 2001):

Low-Risk patients have localized disease and generally surgical excision is the treatment of choice, with adjuvant chemotherapy. Most patients with 4S stage disease experience spontaneous regression and follow-up wait and see or surgery is often all that is needed.

Intermediate Risk: Generally these patients need surgery and multiagent chemotherapy.

High Risk: Patients need an induction multiagent chemotherapy followed by surgery and radiotherapy to primary tumor site and then followed by consolidation chemotherapy and peripheral blood stem rescue.

Induction chemotherapy is done with alkylating agents, platinum, and anthracyclines or topoisomerase II inhibitors. Consolidation myeloablative chemotherapy is done with etoposide, carboplatin, and mephalan. Control of minimal residual disease is done with 13-cis retinoic acid that causes differentiation of neuroblasts.

Future therapies are being studied like aurora kinase inhibitors, antiangiogenic agents, histone deacetylase, and therapeutic metaiodobenzilguanidine (MIBG).

- **Outcome**

This tumor is a challenge to clinicians and researchers as it represents a spectrum of tumors that range from excellent prognosis to highly malignant and lethal tumors. Some of these tumors have excellent prognosis even without treatment. With time, they can maturate or experience spontaneous regression. Others, on the contrary, stay immature despite aggressive therapy, leading the patient to fatal tumor progression. Unfortunately, most tumors are diagnosed late in its history and approximately 70–80% of patients older than 18 months present with metastatic disease. In a small proportion of infant's neuroblastomas with a 4S stage, prognosis is excellent. These patients have a typical presentation and "metastatic pattern" that is most important to recognize as this tumors frequently experience spontaneous regression. These patients are generally younger than 6 months; they present small primary tumors and have metastatic lesions in the liver, skin, and in the bone marrow.

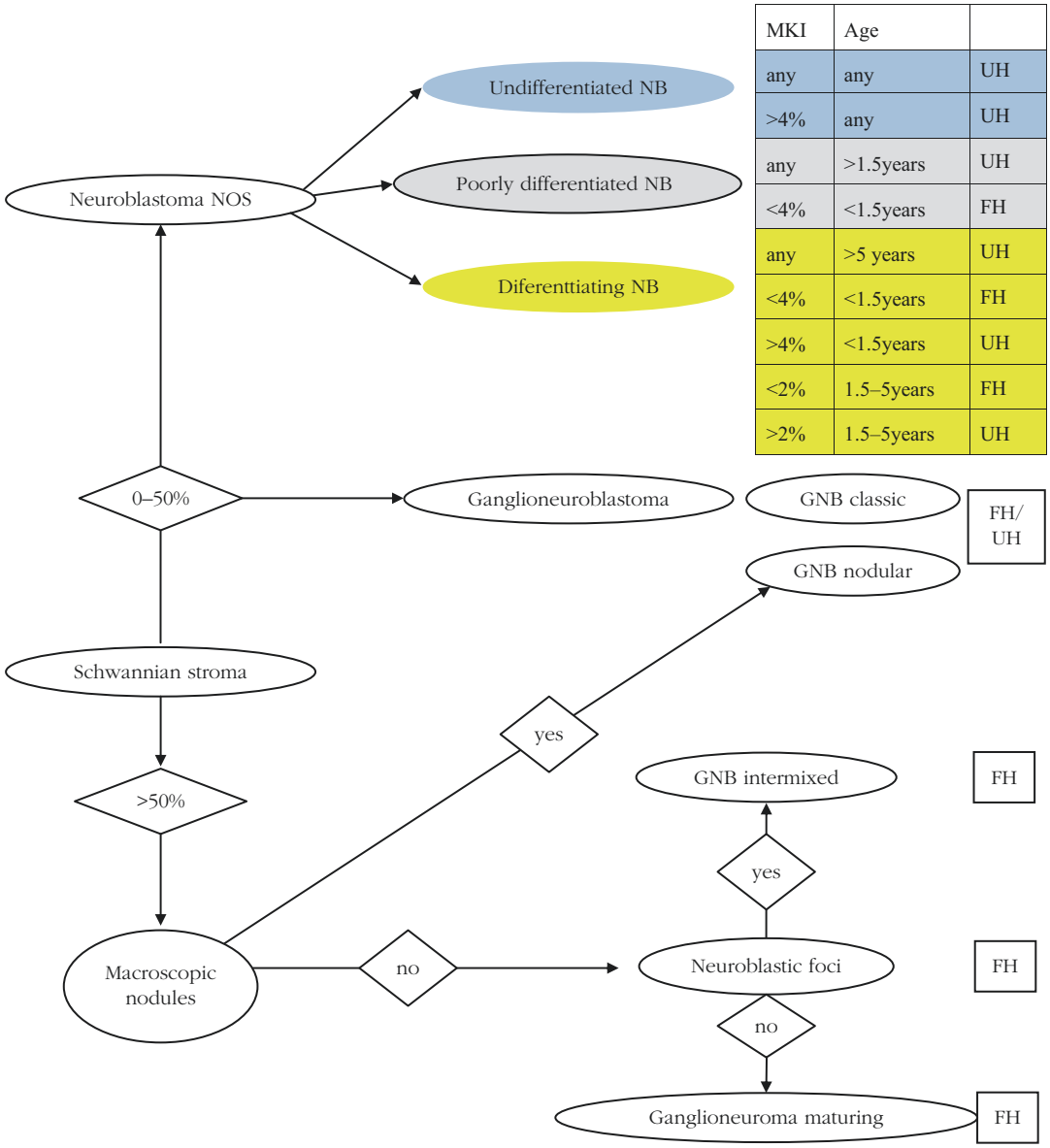
Survival depends on the patient age at diagnosis, clinical stage, location, tumor histology, and biologic variables. In 95% of the cases, elevated levels of catecholamine's in urine are present. Laboratorial markers of bad prognosis are: elevated ferritin, serum lactate dehydrogenase, and neuron-specific enolase levels.

Children less than 1 year are associated with a 90% of 5-year survival, while those between 1 and 4 years show 68% of 5-year survival. Over this age, values drop to 52% of 5-year survival.

Staging neuroblastomas is done according to the International Neuroblastoma Staging System (INSS) (Scheme 1).

The knowledge of molecular markers affecting tumor behavior and patient outcome has permitted a more accurate approach in the treatment and prognostic evaluation of the patients.

Numerous biologic factors influence prognosis. From all, amplification of the N-myc myelocytomatosis viral related oncogene (MYCN) is the most determinant biologic marker. MYCN is overexpressed in approximately 25% of neuroblastomas, it is associated with advanced stage disease and it confers rapid tumor progression. H-Ras has the opposite effect correlating with low-stages and good prognosis. Besides oncogene MYCN, other tumor suppressor genes confer bad prognosis, namely, del 1p, allelic losses of 11q, 14q, and gains on chromosome 17q. DNA index also correlates with response to therapy. Children with hyperdiploid tumors have better response to therapies. Other less important biologic factors are transforming tyrosine kinase protein (TrkA) and TrkB tyrosine kinase or BDNF/NT-3 growth factors receptor (TRKB), CD44, and multidrug resistance protein (MRP).



MKI	Age	
any	any	UH
>4%	any	UH
any	>1.5years	UH
<4%	<1.5years	FH
any	>5 years	UH
<4%	<1.5years	FH
>4%	<1.5years	UH
<2%	1.5–5years	FH
>2%	1.5–5years	UH

Neuroblastoma, Cytological Findings, Scheme 1 Representation of the International Neuroblastoma Pathology Classification. *NB* neuroblastoma, *GNB* ganglioneuroblastoma, *FH* favorable histology, *UH* unfavorable histology

Macroscopy

Macroscopic appearance depends on the percentage of neuroblastomatous component (hemorrhagic and necrotic aspect) versus ganglioneuromatous component (white and firm appearance). Generally, a multinodular aspect with necrotic foci and calcifications is seen.

Microscopy

Tumors are classified according to Risk Groups – see Table 1.

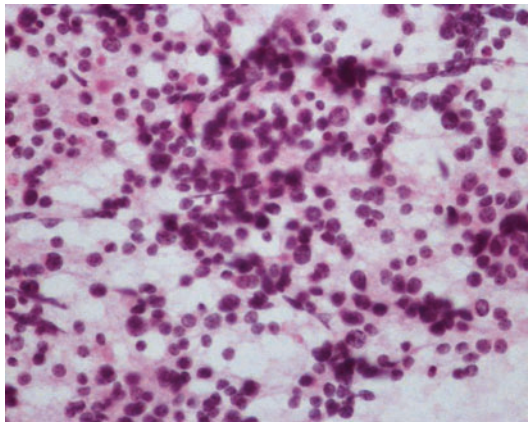
Neuroblastoma manifests as a spectrum of three histological patterns, ranging from neuroblastoma to ganglioneuroblastoma and ganglioneuroma, based on the degree of tumor

Neuroblastoma, Cytological Findings, Table 1 INSS – International neuroblastomas staging system

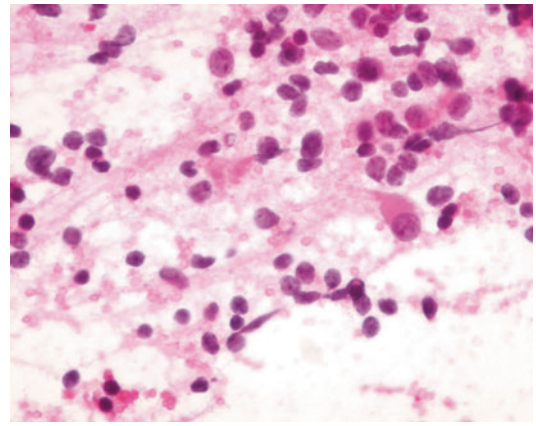
Stage 1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to or removed with primary tumor can be positive)
Stage 2A	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically
Stage 2B	Localized tumor with or without incomplete gross excision; representative ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes negative microscopically.
Stage 3	Unresectable tumor infiltration across the midline defined as the vertebral column with or without regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement.
Stage 4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined in stage 4S).
Stage 4S	Localized primary tumor (as defined in stage 1,2A, and 2B) with dissemination limited to skin, liver, and bone marrow (<10% of the total nucleated cells identified as malignant on bone marrow aspiration smear or biopsy); more extensive bone marrow involvement is considered as stage 4 (limited to infants <1 year of age).

cell differentiation and in the proportion of neuroblastomatous vs. ganglioneuromatous component. Neuroblastoma is composed essentially by two cytological pattern types: the neuroblasts (neuroblastomatous component) and the Schwann cell a mesenchymal cell that creates organized strands that separate nodules of neuroblasts and ganglion cells in maturation (ganglioneuromatous component). Neuroblasts in neuroblastomas can be present in different stages of maturation conferring the tumor a more or less differentiated pattern. Immature neuroblasts are small round blue uncommitted cells with neuroendocrine/neuroepithelial-type chromatin ("salt and pepper") and long neuropil. These cells can mature and gain cytoplasm and nucleoli, transforming themselves in ganglion cells. In the more undifferentiated form of this component, cells have an uncommitted appearance of small round blue cells and arrange themselves in a solid pattern. Differential diagnosis with other small round cell tumors is difficult. Progression to maturity leads to a nodular pattern with delicate "fibrous" septa and the formation of Homer-Wright rosettes. When the ganglioneuromatous component becomes predominant (more than 50% of the tumor), the tumor is called ganglioneuroblastoma (see Table 1 below), and if it represents the only component, with few scattered maturing or mature ganglionary cells, then the tumor is called as ganglioneuroma maturing or mature respectively. The International Neuroblastoma Pathology Classification (INPC) is based on Shimada classification with the advantage that it introduces age as a parameter. This classification is based on the amount of stroma, neuroblastic differentiation, Mitosis-karyorrhexis index of neuroblastoma (MKI) and age and it classifies neuroblastomas in favorable and unfavorable histology – see schema below. In this classification the presence of less or more than 50% of ganglioneuromatous component differentiates neuroblastomas and ganglioneuroblastoma respectively. Neuroblastoma is then subclassified into **undifferentiated**, **poorly differentiated**, and **differentiating** according to the absence, or presence of neuropil and the presence of null, less than 5% or more than 5% of differentiating neuroblasts respectively. Ganglioneuroblastoma is then a stroma predominant tumor, where neuroblasts appear in different stages of maturation as well as maturing ganglion cells. Ganglioneuroma represents the mature edge of the spectrum. In this tumor, the ganglioneuromatous component predominates (Ganglioneuroma maturing) or is the only component (Ganglioneuroma mature). In the maturing subtype, scattered differentiating neuroblasts or maturing ganglion cells are still admitted. The mature subtype is composed only of mature elements (ganglion cells and Schwann cells).

In the INPC classification, microscopic characteristics are conjugated together with age (less than 1.5 years; 1.5–5 years, and more than



Neuroblastoma, Cytological Findings, Fig. 1 Neuroblastoma NOS – Small round cell tumor. The neoplastic cell lie dispersed in a finely fibrillary stroma. Neuroblasts show a moderate anisonucleosis, reflecting their different grade maturation (H&E 400x)



Neuroblastoma, Cytological Findings, Fig. 2 Neuroblastoma NOS – neuroblasts look completely immature and uncommitted cells with high nuclear/cytoplasmic ratio, round nuclei with coarse "salt and pepper chromatin" and inconspicuous nucleoli. Others in different stages of maturation, gain progressively in nucleoli and moderate eccentric eosinophilic cytoplasm (H&E 400x)

5 years), and mitosis-karyorrhectic index (MKI) (below 2%; 2–4% and more than 4%) in order to classify neuroblastomas in favorable and unfavorable histology – see Table 1 below (Mossé et al. 2008).

A specific subtype nodular ganglioneuroblastoma was created for those tumors composed of at least two macroscopic different biological clones. Grossly in these tumors one or more neuroblastic nodules can be macroscopically identified, inside a stroma predominant tumor.

Staging is established by the International neuroblastomas staging system (INSS) – see Scheme 1.

In cytology, the distinction between the different types of Neuroblastoma/ganglioneuroblastoma (INPC classification) is impossible as it depends on the architecture and on an exhaustive sample of the tumor. The diagnosis of neuroblastoma no otherwise specified (NOS) should be done in cytology whenever this entity is recognized. The component most easily detected in smears of neuroblastoma is the neuroblastomatous, immature, component (Figs. 1 and 2). Typically neuroblasts are small round blue cells and appear in the same smear in different stages of maturation (Fig. 2). While some neuroblasts look completely immature and uncommitted cells with high nuclear/cytoplasmic ratio, round nuclei with coarse "salt and pepper chromatin" and inconspicuous nucleoli, others in different stages of maturation, gain progressively in nucleoli and moderate eccentric eosinophilic cytoplasm (Fig. 2). In undifferentiated neuroblastomas, the uncommitted cells are the predominant component (Fig. 1). The cells lay single in a necrotic/apoptotic background. In poorly differentiated and differentiating neuroblastomas, a more polymorphic population is present with neuroblasts in different stages of maturation. In these subtypes, the cells lay single in a fibrillary background or in loose aggregates, with nuclear moulding and sometimes Homer Wright rosettes. Necrosis and apoptosis is a common feature in all neuroblastomas. The presence of a fibrillary background indicates that at least you are in the presence of a poorly differentiated neuroblastoma. Calcifications are characteristic of neuroblastomas, radiologists identify them as a reliable mark in the

diagnosis of this entity (except for synovial sarcoma), and they can be seen in smears.

The ganglioneuromatous (GNR) component gives to the operator of the fine needle aspiration, a hardness feeling during puncture. Tumors where the GNR component predominates generally give hypocellular smears with scarce ganglion cells. These cells are easy to recognize as they are large cells with big eccentric nuclei and prominent eosinophilic nucleoli. Their cytoplasm is abundant and eosinophilic. In ganglioneuroblastomas, the cytological picture is that of moderately to poorly cellular smear with scarce or no necrosis, large single ganglion cells, and some immature dispersed neuroblasts. Schwann cells are rarely aspirated and appear isolated in the background as spindle cells or nude elongated nuclei. Ganglioneuromas give rise to hypocellular smears and are very hard lesions at biopsy. With luck we will be able to visualize scarce dispersed ganglion cells. This type of cytological picture, however, does not guarantee that you are not in the presence of a ganglioneuroblastoma deficiently sampled.

Immunophenotype

Immunostains can help in the diagnosis of neuroblastomas and this tumor is characterized by the immunoexpression of neuroendocrine and neural markers like **C**luster **D**ifferentiation CD56 **N**eural **C**ell **A**dhesion **M**olecule (NCAM), synaptophysin, chromogranin A, neuron-specific enolase (NSE), ganglioside GD2 (only on frozen material), and tyrosine hydroxylase. In cytology and in order to differentiate this entity from other small round cell tumors, it is essential you get acquainted with the classical morphologic aspect of neuroblasts. Although neuroblastomas have a characteristic neuroendocrine immunostaining profile, as described above, these stains do not differentiate it from tumors like rhabdomyosarcoma, desmoplastic small cell tumor, Primitive Neuroectodermal Embryogenic Tumor (PNET), rhabdoid tumor, or even undifferentiated synovial sarcoma. To solve this problem, you should use a more extensive immunostaining panel including, CD99, desmin, myogenin, and vimentin that are usually negative in neuroblastoma.

Molecular Features

In 2% of the cases a familiar story is found, suggesting an autosomal dominant inheritance with incomplete penetrance. These cases seem to be associated to a germline mutation of ALK tyrosine kinase gene (Mossé et al. 2008). **A**naplastic **L**ymphoma **K**inase (ALK) amplification is detected in 15% of primary neuroblastomas with myelocytomatosis viral-related oncogene (MYCN) amplification, and has also been associated to bad prognosis (Ambros et al. 2009).

- Patients with polymorphisms at chromosome 6p22 seem to have a higher susceptibility to neuroblastomas. However, the molecular events that are in the genesis of neuroblastomas are still unknown.
- DNA ploidy is another molecular prognostic marker. Hyperdiploid tumors have good prognosis whereas diploid tumors not only tend to have a poor prognosis, but also are associated with other High-Risk genetic aberrations, such as 17q gain and *MYCN* amplification. Ploidy seems to be response predictor in those patients younger than 18 months with advanced stage and no MYCN amplification.
- MYCN amplification is detected in 20–30% of primary neuroblastomas and it confers these patients a tumor progression and a fatal outcome despite intensive therapy. It is more frequently amplified in advanced stage tumors (40%) but it is also found in 5–10% of early stage and 4S tumors. The detection of MYCN amplification is important as it stratifies the patient in a High-Risk group and consequently determines a more aggressive therapy. The meaning of this amplification in 4S stage

tumors and in localized tumors is yet to be evaluated. According to the Children's Oncology Group (COG), more than 10 copies of the gene must be identified to have a clinical value of adversity. Coamplifications of other genes like ALK, ATP-dependent RNA helicase DDX1, and neuroblastoma amplified gene (NAG) can be present (Wimmer et al. 1999).

- Deletion of chromosomal region 1p36.3 characterizes 30–40% of neuroblastomas conferring a bad prognosis. It can be associated with MYCN amplification but can also exist in its absence.
- Unbalanced chromosome 17q gain is a common molecular alteration on neuroblastomas (about 60%), associated with bad prognosis and metastasis.
- 11q deletions are associated with 17 q gains and inversely associated to MYCN amplification and represent a powerful biomarker in cases with no MYCN amplifications.

Tyrosine **K**inase TRKA and TRKB are neurotrophic receptors also associated to prognosis in neuroblastomas. While TRKA is associated to good prognosis and no MYCN amplification, TRKB is associated to tumor progression and resistance to chemotherapy (Peuchmaur et al. 2003; Lavoie et al. 2005).

- Apoptosis pathways have been studied in neuroblastomas. Although P53 seems to have no interference in neuroblastomas, it seems that other apoptotic related genes, such as BCL-2 and caspase-8, are intimately related to prognoses of neuroblastomas.
- Multidrug resistance P-glycoprotein (MRP) is overexpressed in MYCN amplified neuroblastomas, correlating with advanced stages and unfavorable histology.
- Angiogenic factors: Neuroblastomas widely disseminated with MYCN amplification and unfavorable histology have increased expression of vascular endothelial growth factor, basic fibroblastic growth factor, platelet-derived growth factor A, and integrins, which are markers of active angiogenesis. Curiously, stroma-rich neuroblastomas (ganglioneuroblastomas and ganglioneuromas), and numerous angiogenic inhibitors secreted by Schwann cells appear to maintain the inhibitory phenotype.

Differential Diagnosis

Differential diagnosis should be performed with most of small round cell tumors that can appear in similar location. Clinical setting as well as immunohistochemistry is essential to differentiate these tumors from lymphoma, alveolar rhabdomyosarcoma, desmoplastic small cell tumor, PNET, rhabdoid tumor, or even undifferentiated synovial sarcoma.

These entities share a similar cytological pattern, characterized by a monotonous population of single, round blue cells. Minor morphological clues should call the attention of an experienced pathologist. Most daily routine neuroblastomas appear as poorly differentiated neuroblastomas. Typically, in these smears, the background is fibrillary, with apoptotic nuclei and neuroblasts with a salt and pepper chromatin. Normally, neuroblasts are found in different stages of maturation, giving the impression of a heterogeneous cell population. A variability of cellular size, cytoplasm quantity, and nucleoli characterize this neoplastic population. The great challenge is in undifferentiated neuroblastoma where no clue is available to suspect of the diagnosis. In these cases a rationale for exclusion must be conducted and supplemented by immunocytochemistry or molecular analysis to exclude other diagnoses. Tigroid background, neoplastic cells with finely dispersed chromatin, the presence of tadpole or racquet cells should point out to a rhabdomyosarcoma. The presence of lymphoglandular bodies should alert to a possible lymphoma. In PNET tumors, a tigroid background can also be appreciated. A dimorphic dark (apoptotic) and light neoplastic population is seen. The neoplastic cells, when preserved, show minute small heterogeneous glycogen vacuoles.

In most instances, immunocytochemistry is essential for achieving the diagnosis; however, immunocytochemistry is not always concluding, and some of these entities even share antigenic determinants. Malignant small round cell tumors tend to be characterized by specific balanced translocations, whose detection can be useful for achieving the correct diagnosis. Thus, it is essential, during FNB procedure, to assure that enough material is obtained and saved for further genetic studies.

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Nodular Hyperplasia of Prostate, Cytological Findings

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Synonyms

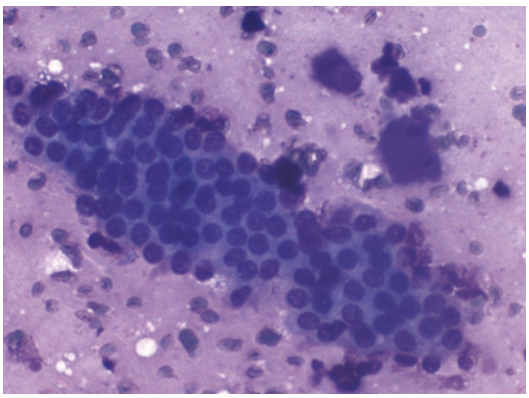
Adenofibromyomatous hyperplasia; Benign enlargement of the prostate; Benign prostatic hypertrophy

Definition

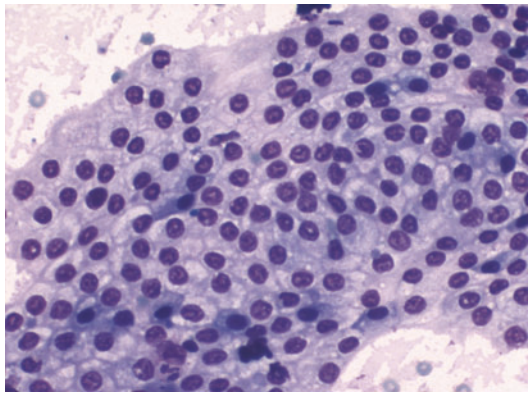
It is a benign enlargement of the prostate due to increased number of cells of both prostatic stromal and epithelial components (Fig. 1).

Clinical Features

- **Incidence**
  It is seen in a majority of elderly men.
- **Age**
  Most are 50 years or older.



Nodular Hyperplasia of Prostate, Cytological Findings, Fig. 1 Benign prostate hyperplasia: monomorphic cells with round nuclei with distinct cytoplasm in a tissue fragment. Concrement can be seen as irregular structures. MGG



Nodular Hyperplasia of Prostate, Cytological Findings, Fig. 2 Benign prostate hyperplasia: a tissue fragment with hyperplastic cells with monomorphic nuclei and clear distinct cytoplasm forming a honey comb pattern. MGG

- **Sex**
  It is an exclusive male disease.
- **Treatment**
  Surgery, cryotherapy, laser therapy, and hormonal therapy.
- **Outcome**
  Usually progressive but symptoms vary considerably.

Microscopy

Monomorphic epithelial cells with well-defined cytoplasm, often with granules, in honeycomb clusters Amorphous protein background often with macrophages (Fig. 2).

Differential Diagnosis

Well-differentiated adenocarcinoma.

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Nodular Hyperplasia of Thyroid, Cytological Findings

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Synonyms

Adenomatous/adenomatoïd nodule; Hyperplastic nodule; Multinodular goiter

Definition

The term “goiter” refers to any enlargement of the thyroid gland and is considered as a non-neoplastic process, usually benign, essentially due to iodine deficiency, but sometimes of unknown etiology. The term “goiter” includes both either a thyroid gland deformed by numerous nodules of varying sizes or a diffuse enlargement of the gland. The nodular goiters are histologically represented by two different types of nodules: the **colloid nodules**, related with accumulation of the colloid within the follicles and the **hyperplastic** (**adenomatoid**) **nodules**, characterized by a hyperplasia of the follicular cells.

Clinical Features

- **Incidence**
  Nodular goiters represent one of the most frequent diseases in the world with a prevalence from 1% to 10%, depending on geographic locations. Goiters are more frequent in mountains than in the low lands and on the sea-side. In the United States, there is an estimated annual incidence rate of 0.1% per year.
- **Age**
  The goiter increases with age.
- **Sex**
  It is more frequent in women.
- **Site**
  Goiter is a term which means that the gland is entirely modified.
- **Treatment**
  Treatment depends on the clinical data; in case of iodine deficiency, iodine-rich foods may be introduced into the diet, but it is often not sufficient. Hormone substitutive therapy is required in case of hypothyroidism. In case of hyperthyroidism, drugs blocking the gland are necessary. Goiter without any hormonal trouble may benefit either from surgery or from radioiodine therapy when the nodules are voluminous and troublesome, eventually compressive. Mostly, considering that nodular goiter is a non-neoplastic disease, it does not require surgery.
- **Outcome**
  Usually excellent since it is a benign disease. Extreme hyper- or hypothyroidisms forcing immediate treatment are rare. On the counterpart there is about a 5–7% risk of cancer. Depending on the studies, this risk is considered lower than the risk of cancer in a single nodule or equal.

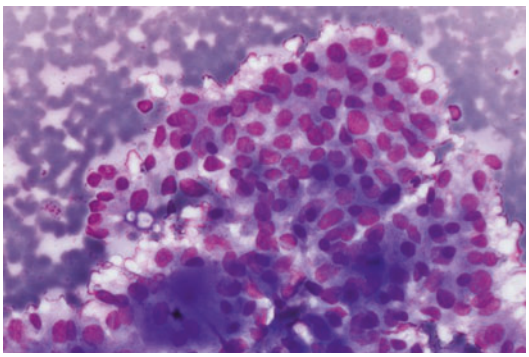
Macroscopy

Lobectomy as well as near-total thyroidectomy may be examined. The number of nodules varies a lot, from 3 to 5 nodules per lobe to more than 20 nodules. The nodules also vary in size from 4 mm to 5 mm to 3 cm or more; finally, the nodules show different aspects, depending on the quantity of colloid and the existence or not of hemorrhagic areas. Hyperplastic nodules are usually well limited, may be with a thin capsule and with few colloid.

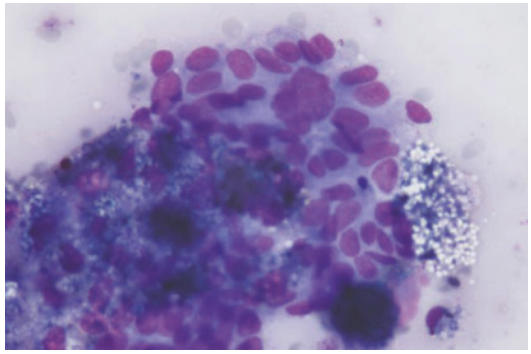
Microscopy

In this entry, description concerns only the hyperplastic nodules; the colloid nodules are described in another one (see entry "▶ Colloid Goiter, Cytological Findings"). Hyperplastic nodules are highly cellular on fine needle aspiration, made up of: (1) numerous sheets of follicular cells sometimes monolayered but more often showing some microfollicular arrangements or even some papillary features; nuclear overlapping is common but usually the nuclei are regular; (2) more or less colloid; (3) Histiocytes with or without hemosiderin; (4) cells in which nuclei are sometimes enlarged, with variable degrees of anisokaryosis; but the chromatin does not change. Grooved nuclei have been described; (5) some oncocytes may be present (Figs. 1 and 2).

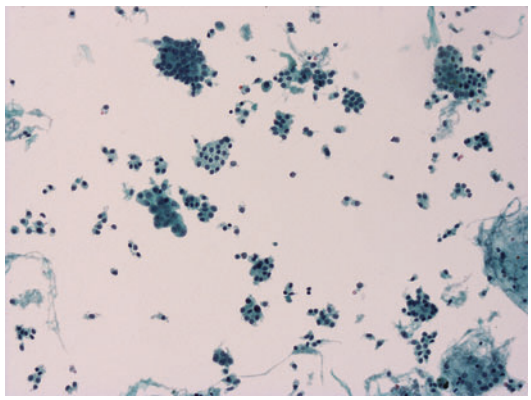
In liquid-based cytology, essentially with the Hologic® technique, there are some significant changes mainly due to the alcoholic fixative:



Nodular Hyperplasia of Thyroid, Cytological Findings, Fig. 1 Sheet of follicular cells with some overlapping and slightly enlarged nuclei (conventional smear; MGG staining ×40)



Nodular Hyperplasia of Thyroid, Cytological Findings, Fig. 2 Sheet of follicular cells mixed with histiocytes. The follicular cells have enlarged, sometimes grooved nuclei (conventional smear; MGG staining ×40)



Nodular Hyperplasia of Thyroid, Cytological Findings, Fig. 3 Hypercellular material with numerous sheets of cells, histiocytes, and no colloid (LBC; Hologic®; Papanicolaou staining ×25)

(1) the colloid appears either like dense dark blue droplets or like dark blue-orange in the center droplets; but often, due to the technique, the colloid may lack; (2) follicular cells show the same arrangements than on conventional smears. The cytoplasm is scant, blue pale, and the nuclei are intensively shrunk and hyperchromatic; (3) Histiocytes may be observed; (4) Oncocytes are also modified with eccentric, dense, sometimes irregular nuclei and with a more homogeneous cytoplasm than on conventional slides (Fig. 3).

Immunophenotype

The follicular cells are positive for thyroglobulin and TTF1. They are negative or weakly positive for CK19, Galectin-3, and HBME1. Nevertheless, in some cases, a higher positivity may be observed.

Molecular Features

RET/PTC rearrangement has been found in benign nodules, especially in hyperplastic nodules with a rapid size increase. Inversely BRAF V600 mutation is found only in papillary carcinoma and is therefore more specific than RET/PTC

Differential Diagnosis

A diagnosis of hyperplastic nodule is usually easy for skilled cytopathologists. However, due to its high cellularity and to some more frequent follicular arrangements, it may be classified as a "Follicular Lesion of Undetermined Significance" (Bethesda 2009); if some atypia suggestive of papillary carcinoma are added, then even a diagnosis of nodule "Suspicious for malignancy" (Bethesda 2009) may be proposed inducing surgery and a false-positive case. When favored benign, immunocytochemistry may be helpful.

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Non-Hodgkin Lymphomas, Cytological Findings

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Variants

Precursor lymphoid neoplasms (B- or T-phenotype), mature B-cell neoplasms, mature T-cell neoplasms, mature NK-cell neoplasms.

Definition

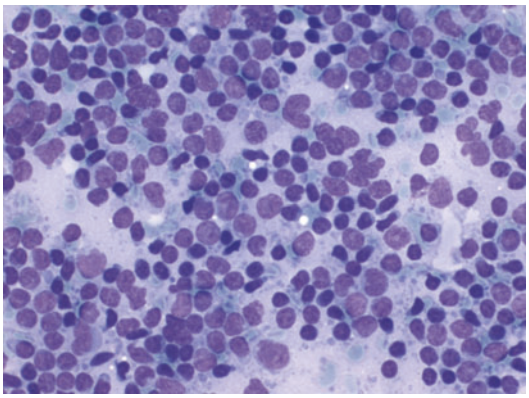
Neoplasms arising from various types of normal lymphoid cells as indicated by morphology, immunophenotype, genetic studies, and clinical features.

Clinical Features

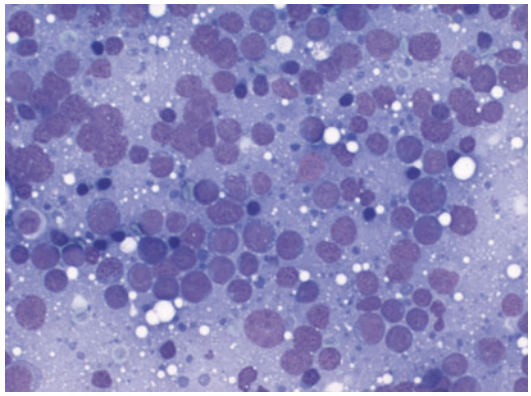
- **Incidence**
  Incidence of non-Hodgkin lymphomas is 14–17/100,000. Various subtypes such as follicular lymphoma and diffuse large B-cell lymphoma together account for 50–60% of lymphomas while other subtypes, e.g., Alk-positive large B-cell lymphoma is very rare.
- **Age**
  The precursor B- and T-cell lymphomas dominate among children and young adults, while mature B- and T-cell neoplasms are commonly seen in middle aged and elderly.
- **Sex**
  The various subtypes show different male: female ratio, but in general, slight male predominance.
- **Site**
  Lymph nodes and bone marrow are by far the most common sites for lymphoma, but almost all organs can be affected.
- **Treatment**
  Multi-agent chemotherapy, monoclonal antibodies, and radiation therapy.
- **Outcome**
  Some high-grade lymphoma can be cured by intensive chemotherapy. In contrast, many low-grade lymphomas are incurable with current therapy although many patients have a long survival time.

Microscopy

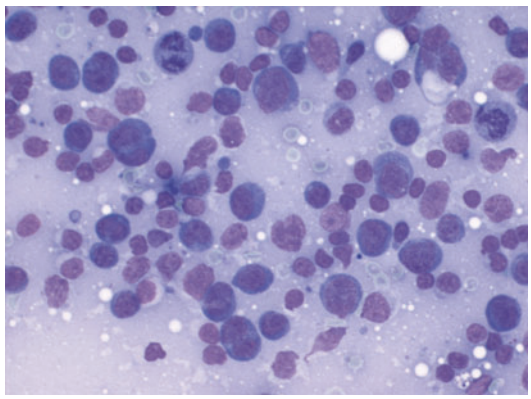
In most lymphomas, one cell type dominates but reactive lymphoid cell can be present. The premature B- and T-cell lymphoma are characterized by small, round immature looking neoplastic cells. The low-grade mature B-cell neoplasms are dominated by small- to medium-sized neoplastic lymphoid cells (Fig. 1). Some subtypes, e.g., follicular lymphoma, may present some neoplastic centroblastic cells in addition to the more



Non-Hodgkin Lymphomas, Cytological Findings, Fig. 1 Non-Hodgkin lymphoma, follicular lymphoma grade 1: A monotonous population of medium-sized lymphoma cells with sparse cytoplasm (centrocytes). Only few large cells of centroblastic type are seen. MGG



Non-Hodgkin Lymphomas, Cytological Findings, Fig. 2 Non-Hodgkin lymphoma, follicular lymphoma grade 3: Large lymphoma cells with round nuclei and distinct cytoplasm (centroblasts) dominate and only few mature cells are present. MGG



Non-Hodgkin Lymphomas, Cytological Findings, Fig. 3 Non-Hodgkin lymphoma, diffuse large cell lymphoma: Immature large polymorphic lymphoma cells with irregular nuclei, often with a distinct nucleolus. Mitotic figures are seen. MGG

common medium-sized neoplastic lymphoid cells (Fig. 2). High-grade B-cell lymphomas are characteristically composed of large cells, with varying degree of pleomorphism (Fig. 3). T-cell lymphomas often show a broad cytologic spectrum of neoplastic cells with numerous reactive lymphocytes and often granulocytes present.

Immunophenotype

The neoplastic cells of B-cell lymphoma express B-cell antigens (e.g., CD19, CD20 and CD79a). In addition, light chain restriction can be demonstrated in most cases. The subtypes of different T-cell lymphoma show expression of various T-cell surface markers such as CD3, CD4, CD5, CD7,and CD8 as well as CD30.

Molecular Features

Most B-cell lymphomas show rearrangement of IG genes. In addition, translocations are common. T-cell lymphomas often show clonal rearrangement of the TCR genes. However, some subtypes, e.g., aggressive NK-cell leukemia/lymphoma, have germ line TCR.

Differential Diagnosis

Various reactive conditions, metastasis from carcinoma and melanoma.

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O

Oligodendroglioma, Cytological Findings

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Synonyms

Glioma

Definition

Neoplasia composed of cells resembling oligodendroglia.

Clinical Features

- **Incidence**
  The incidence has been estimated to be 0,27–0,35 per 100,000 persons and correspond to 5–6% of gliomas.
- **Age**
  The oligodendroglioma has a peak of incidence between 40 and 45 years.
- **Sex**
  Men show slightly greater incidence than women at a ratio of 1.1:1.
- **Site**
  The oligodendrogliomas occur predominantly in the cortex and white matter of the cerebral hemisphere and the frontal lobe involved in 50–65% of cases.
- **Treatment**
  The treatment of patients with oligodendroglioma involves surgery, radiotherapy, and chemotherapy.

Outcome

Patients with oligodendrogliomas have median survival from 4.4 to 9.8 years with survival rates of 38–75% after 5 years and 19–59% after 10 years.

Macroscopy

Oligodendroglioma usually presents as diffuse neoplasm with grayish-pink color, gelatinous consistency with poorly defined margins, and areas of calcification.

Microscopy

In cytological smears oligodendrogliomas are characterized by monotonous sheets or nests and cells (Fig. 1) with clear boundaries and cytoplasmic and perinuclear halo. The nuclei are round

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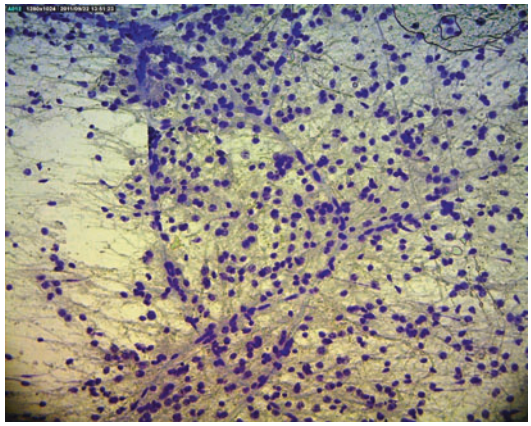
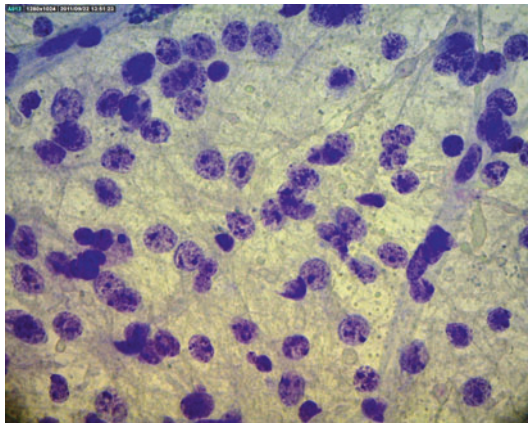


Oligodendroglioma, Cytological Findings, Fig. 1 Oligodendroglioma-note smear with high cellularity in nests septate by delicate blood vessels (*toluidine blue*) (Note: Source of the figures of the authors)



Oligodendroglioma, Cytological Findings, Fig. 2 Oligodendroglioma-note cells with monotone nuclei round with delicate chromatin and regular nuclear membrane (*toluidine blue*) (Note: Source of the figures of the authors)

and regular with delicate chromatin and regular nuclear membrane (Fig. 2). Mitoses and foci of calcification may be observed. In areas can be visualized septation of cell nests by vasculature. In malignant cases there will be necrosis and vascular proliferation.

Immunophenotype

The immunophenotype of oligodendroglioma includes reactivities for anti-Leu 7, CD57, and microtubule-associated protein 2 (MAP2).

Molecular Features

The molecular alterations observed in oligodendroglioma are translocation between chromosomes 1 and 19 with co-deletion of 1p and 19q.

Differential Diagnosis

Oligodendrogliomas may be confused with other tumors with small round cells like pituitary adenomas, pineal germinoma, and lymphomas.

Pituitary adenomas show nuclear sheets and cohesive cells and nuclei with multiple nucleoli. Pineal germinomas present large cells than oligodendroglioma with abundant pale cytoplasm and large vesicular nucleus with prominent nucleolus and associated population of lymphocytes. In lymphomas there is a wider spectrum of nuclear morphology with frequent mitoses. Lymphomas often contain thick-walled blood vessels which are infiltrated by tumor cells.

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Orbit Cytology, General Aspects

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Description

The orbit is a bony cavity with medial and lateral walls, roof, and floor, which contains the globe, the lachrymal glands in the upper lateral parts, and fibrous and fatty tissue in the posterior cone. The orbit also contains the orbital muscles, branches of the cranial nerves, and a small number of lymphocytes and vessels. Although the globe is strongly protected by the bony consistency of the orbit, intracranial expansive processes or masses of the sinuses may invade the orbital cavity by passing through the foramen caecum or interrupting the thin bony laminae which divide the sphenoidal sinuses from the orbit medially. Furthermore, inflammatory processes of the maxillary sinus, even from dental implants, can permeate the orbital floor. Orbital masses may cause eyelid ptosis, puruloid secretions, dysfunction of ocular mobility, diplopia, and proptosis as in Graves' disease. They may be sampled by FNC using 21–23 gauge needles under US or CT guidance or without guidance by a skilled orbital ophthalmologist. The approach may be transcutaneous at the base of the eyelid. The ophthalmologist places a finger on the globe, exerts light pressure downwards or upwards with the fingertip, and inserts the needle parallel to the nail through the eyelid skin. In this way the roof, floor, and upper lateral area may be reached and sufficiently large masses may be easily aspirated. The inadequacy rate and possible complications such as hemorrhages represent the main disadvantages of the procedure. Considering the limited possibility of movement of the needle in this area and the fibrous matrix, which is a feature of many orbital expansive processes, the use of a larger needle could increase the harvest of cells but could also increase the risk of complications. In our experience, aspiration is definitely more effective than capillary cytology, and a 23 gauge needle is fine enough to reduce possible complications but wide enough to collect cells.

Cross-References

▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
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▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
▶ Conjunctiva Cytology, General Aspects
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▶ Conjunctival Papilloma, Cytological Findings
▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
▶ Cornea Cytology
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▶ Eyelids Cytology, General Aspects
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▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
▶ Meningioma, Cytological Findings

- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
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Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

Orbital cysts are a heterogeneous group of cystic lesions that differentiate for clinical and pathological findings. The main orbital cysts are mucocele and dermoid cyst.

Clinical Features

- **Incidence**
  Common.
- **Age**
  Any age, dermoid cysts from infancy to adult life.
- **Sex**
  No gender predilection.
- **Site**
  Orbit.
- **Treatment**
  Surgery.
- **Outcome**
  Good prognosis.

Macroscopy

A mucocele is a mucoid or debris-filled cyst lined by mucus-producing cells, which may arise from the lachrymal glands and the lachrymal sac or even as result of chronic inflammation of the ethmoid sinus which may erode the wall expanding into the orbit.

Microscopy

As with its salivary counterpart, the aspiration of mucoid material and bland mucus-producing cells should not automatically lead to a diagnosis of a benign cystic lesion such as a mucocele. An extremely well-differentiated, low-grade ▶ mucoepidermoid carcinoma and a mucocele may show similar cytological features; therefore, in these cases, a diagnosis in which both possibilities are proposed should be considered.

Orbital capsulated cystic lesions generally require surgical treatment; therefore, FNC is rarely requested. Dermoid cysts are the most frequent; FNC may show squamous cells or just debris. Papanicolaou stain may be helpful in recognizing the presence of keratin. Because of the risk of leakage with consequent foreign body reaction, fine needles and gentle movements are advisable.

Differential Diagnosis

Mucocele: low-grade mucoepidermoid carcinoma

Cross-References

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Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

Lymphoid tumor.

Clinical Features

- **Incidence**
  The most frequent orbital malignancy, whereas systemic lymphomas rarely involve the orbit.
- **Age**
  Middle old age.
- **Sex**
  Incidence rate slightly higher for males than for females.
- **Site**
  Orbit.
- **Treatment**
  Radiotherapy and chemotherapy.
- **Outcome**
  Survival rate after 5 years – 60%.

Macroscopy

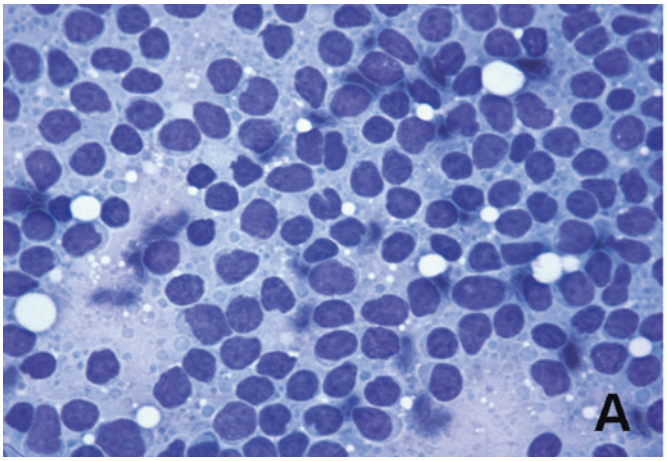
Primary lymphomas may occur in the lachrymal glands, and they may arise "ex novo" or as a result of the transformation of an inflammatory pseudotumor.

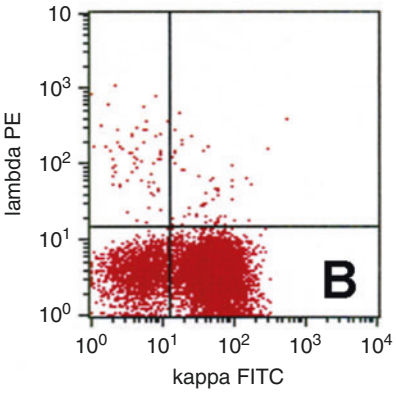
Microscopy

Lymphomas arising from lachrymal glands are mainly represented by small-cell, monocytoid B-lymphocytic lymphomas, like the MALT lymphoma at other extra-nodal sites, but large-cell lymphoma may also occur. Smears generally show a monomorphous population of small lymphocytes with dense chromatin but without nucleoli or chromocenters (Fig. 1a); concomitant fibrosis and cell fragility may damage the cells, making smear interpretation more difficult. In other cases, smears are generally richer, better preserved, and more polymorphous. Follicular center cells in different stages of maturation are intermingled with small "dark" lymphocytes and plasma cells, giving the smear a relatively more polymorphous appearance. Large-cell lymphomas may arise ex novo or as the result of the evolution of a preexisting low-grade lymphoid process.

Immunophenotype

In the presence of large atypical lymphoid cells, the immunocytochemical demonstration of their lymphoid origin may be sufficient for a definitive diagnosis of lymphoma. The combination of FC phenotypization (Fig. 1b) and FNC (FNC/FC) is mainly effective in discriminating between





Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings, Fig. 1 (**a**) Orbital lymphoma FNC: smear showing monomorphous medium-sized cells with irregularities of the nuclear membrane (Diff-Quik stain 430×). (**b**) Corresponding flow cytometry evaluation showing kappa light chain restriction

benign reactive hyperplasia (BRH) and small-cell NHL; conversely, FNC smear coupled with ICC is more effective in the diagnosis of large-cell NHL. Therefore, on-site evaluation may be useful in the choice of the most useful procedure, according to the cytological features, to the management of residual material and or additional FNC.

Molecular Features

Immunoglobulin heavy chain (IGH) and T-cell receptors (TCR) rearrangements are the hallmark of B- and T-cell lymphomas, respectively. These rearrangements can be detected by different molecular procedures such as polymerase chain reaction (PCR). B-cell-differentiated non-Hodgkin lymphoma often shows specific translocations that can be detected by fluorescence in situ hybridization (FISH).

Differential Diagnosis

Inflammatory pseudotumor and lymphoproliferative disorders.

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Orbital Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Description

Malignant orbital tumors may arise from any of the anatomical components of the orbital region; they include tumors of the posterior chamber of the globe such as retinoblastoma, ▶ melanoma, melanocytoma and astrocytoma, malignant lymphomas, sarcomas including pediatric tumors, tumors invading the orbit from adjacent sites, lachrymal gland tumors, and metastases. In general, the less differentiated these tumors are, the less possible is an accurate cytological diagnosis, especially without the aid of ancillary techniques. Considering the wide range of possible lesions and the unlikelihood of obtaining sufficient material, it would be wise to collect all the clinical and instrumental information before performing an orbital FNC in order to refer to the diagnostic algorithm and choose the appropriate ancillary technique when possible. Imaging techniques have become highly informative and may be conveniently evaluated by cytopathologists too in order to reduce the number of lesions included in the differential diagnosis.

Cross-References

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- Conjunctival Papilloma, Cytological Findings
- Conjunctival Squamous Cell Carcinoma, Cytological Findings
- Cornea Cytology
- Cytology of the Orbit and Ocular Adnexa
- Eyelids Cytology, General Aspects
- Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
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- Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
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- Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings

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Description

Orbital metastases may appear in advanced stages of neoplastic disease or may represent the first clinical evidence of a malignant tumor. Diagnosis may be made using FNC, and the use of ICC may contribute to the identification of the primary tumor, thus, avoiding useless and complex biopsies. Metastases from breast and lung carcinomas are the most frequently observed

although many other histotypes have been described, such as hepatocellular carcinoma, esophageal adenocarcinoma, prostatic carcinoma, chordoma, thymic carcinoma, and various hematological processes including Langerhans cell histiocytosis. Metastatic neuroblastoma may occur in the eye and orbit, generating problems of differential diagnosis with retinoblastoma. Microscopically, Flexner-Wintersteiner rosettes are more frequently associated with retinoblastoma than with neuroblastoma although they are infrequently or not easily detected in cytological samples. Moreover, as the two tumors share the same neuroectodermic origin and hence the same antigenic and ICC pattern, clinical context and imaging are decisive in their differential diagnosis.

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
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- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
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Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings

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Description

Orbital soft tissue tumors are represented by a wide spectrum of lesions almost identical to those found in other anatomical regions. Cytology is rarely used for diagnosis; nonetheless, sporadic reports have documented the cytological features of different soft tissue tumors. As far as bone tumors are concerned, cytological features of the orbital extension of a chondrosarcoma of the nasal cavity and sarcomatous transformation of orbital bone in Paget's disease diagnosed by FNC have been reported. Chondrosarcoma smear is characterized by variable mononuclear and binucleated tumor cells often embedded in myxoid background matrix; cells are generally large with well-defined cytoplasm and rounded or irregular nuclei. Sarcomatous transformation of Paget's disease shows a pleomorphic pattern of malignant cells, either isolated or in small groups with a variable amount of matrix. Cytological presentation is generally nonspecific unless osteoid differentiation is identified. Embryonal rhabdomyosarcoma is the most frequent orbital soft tissue malignancy of infancy. The FNC features are those of a small round tumor cell of infancy; the cytological diagnostic criteria have been previously reported.

Cross-References

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Osteosarcoma, Cytological Findings

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Synonyms

Central osteosarcoma; Chondroblastic osteosarcoma; Classical osteosarcoma; Conventional osteosarcoma; Fibroblastic osteosarcoma; Osteoblastic osteosarcoma

Definition

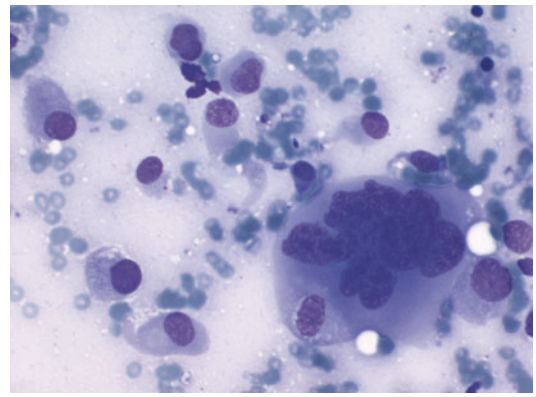
High-grade tumor with osteoid-producing sarcoma cells.

Clinical Features

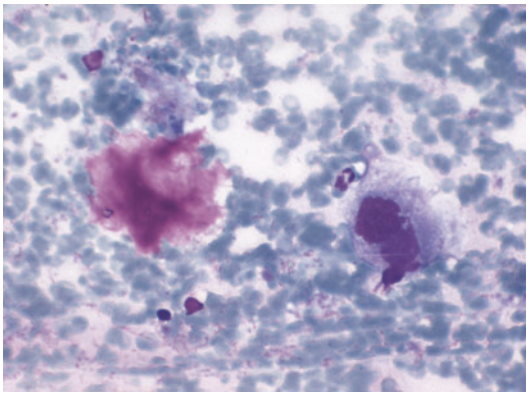
- **Incidence**
  4/1 000 000.
- **Age**
  A majority under 25 years.
- **Sex**
  Males are affected 2–3 times more often than females.
- **Site**
  Meta-diaphysis of long bones.
- **Treatment**
  Chemotherapy, surgery.
- **Outcome**
  Approximately 70% survive.

Microscopy

Pleomorphic anaplastic cells which may be round, spindle shaped, ovoid, and epithelioid. Most tumors are composed of several cell types. Osteoid can be seen as dense intercellular material (pink in MGG) (Figs. 1 and 2).



Osteosarcoma, Cytological Findings, Fig. 1 Osteosarcoma: elongated tumor cells with peripherally located round to oval nuclei with *grey-blue* cytoplasm. One giant tumor cells with multilobated nucleus. MGG



Osteosarcoma, Cytological Findings, Fig. 2 Osteosarcoma: one large tumor cell with an irregular nucleus and *grey-blue* cytoplasm. A fragment of pink osteoid. MGG

Immunophenotype

Vimentin, osteonectin, and CD99-positive. Some cases are immunoreactive for cytokeratin and actin.

Molecular Features

Complex aberrations with various numerical and structural alterations.

Differential Diagnosis

MFH, high-grade chondrosarcoma, high-grade angiosarcoma, metastasis of sarcomatoid carcinoma.

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Ovarian Nonneoplastic Cysts, Cytological Findings

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Definition

The most common nonneoplastic cysts are functional ovarian cysts, i.e., follicular cysts and corpus luteum cysts, which are developed as a result of disturbed ovulation. Follicular cyst results when the dominant follicle fails to release an oocyte and continues to grow or when immature follicle fails to involute. Usually, these cysts produce no symptoms and disappear by themselves within a few months.

Follicular Cyst

Synonyms
Follicle cyst; Follicular cyst

Cytologic Findings

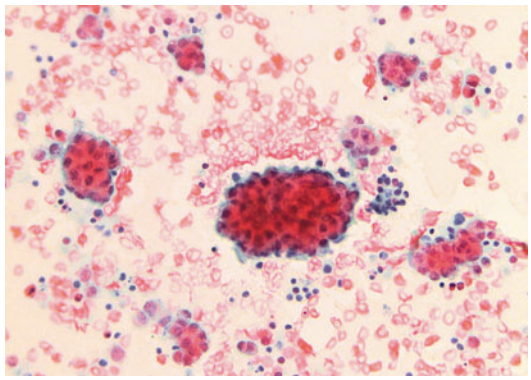
Fluid Aspiration (FNA) *Macroscopically* fluid is clear, yellowish, rarely haemorrhagic (Ivić et al. 1976). *Microscopically* cellular composition from follicular cysts aspirations is diverse, ranging from scanty to abundantly cellular (Greenebaum et al. 1992). The background is finely granular, rich in fibrin.

Two granulosa cell types predominate, depending of follicle stage development (Greenebaum et al. 1992). The first type is a small cell, approximately the size of histiocytes, round or oval in shape, with a round eccentrically placed nucleus, and small rim of distinct basophilic cytoplasm (Figs. 1 and 2) (Ivić et al. 1976). They have granular chromatin, sometimes hyperchromatic with visible chromocenters and one to two micronucleoli. Rarely, cells are performed surrounding a small central luminal space, similar to a Call-Exner body (Fig. 3) (Bibbo et al. 2008). Rarely, an ovum surrounded by granulosa cells may be encountered. The second type of cells is a slightly larger cell with indistinct borders and moderately abundant microvacuolated cytoplasm, consistent with the beginning of the luteinization (Fig. 4). The nuclei have coarsely granular chromatin and mitoses may be observed. The granulosa cells appear isolated, in loose sheets or three-dimensional clusters (Fig. 5). Atypical features include high cellularity, three-dimensional (papillary) clusters, anisonucleosis, an increased nuclear/cytoplasmic ratio of cells, and numerous mitotic figures, sometimes suggesting a malignant granulosa cell tumor. (Ganjei 1995) Knowledge of the clinical history and ultrasound features may be important in preventing diagnostic errors. The presence of ciliated cells or ciliated fragments (ciliated bodies or tufts) oriented the diagnosis toward a cyst with serous-type epithelium (Allias et al. 2000).

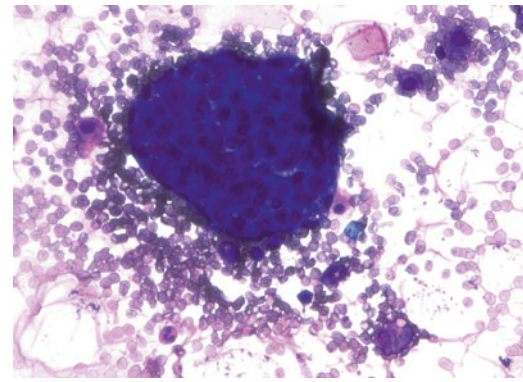
Luteal Cyst

Synonyms

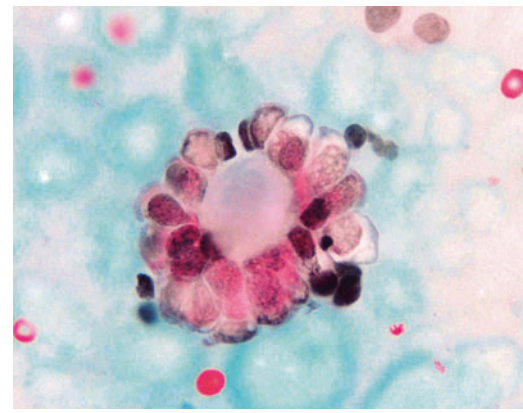
Corpus luteum cyst; Luteal cyst; Luteinized cyst



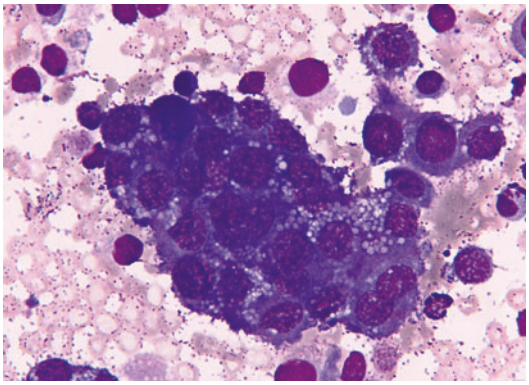
Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 1 The nuclei of granulosa cells are round or oval with coarsely granular chromatin and small rim of distinct basophilic cytoplasm (FNA. Papanicolaou ×400; MGG×400)



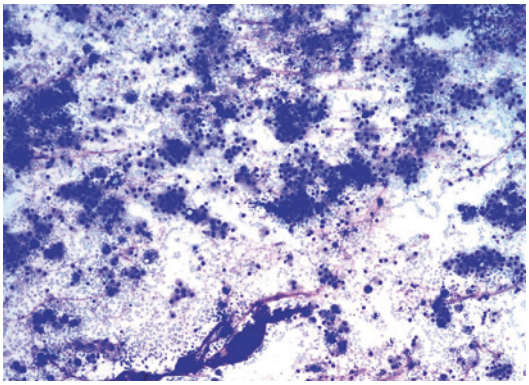
Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 2 The nuclei of granulosa cells are round or oval with coarsely granular chromatin and small rim of distinct basophilic cytoplasm (FNA. Papanicolaou ×400; MGG×400)



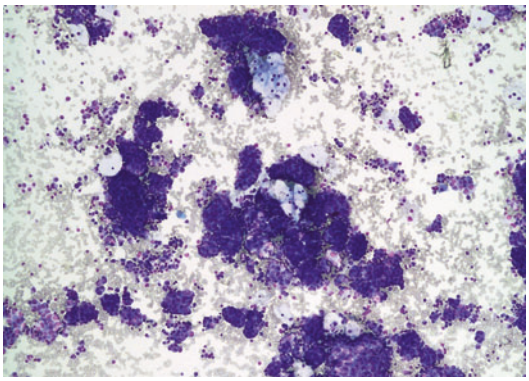
Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 3 Granulosa cells surround a small luminal space, similar to a Call-Exner body (FNA. Papanicolaou ×1000)



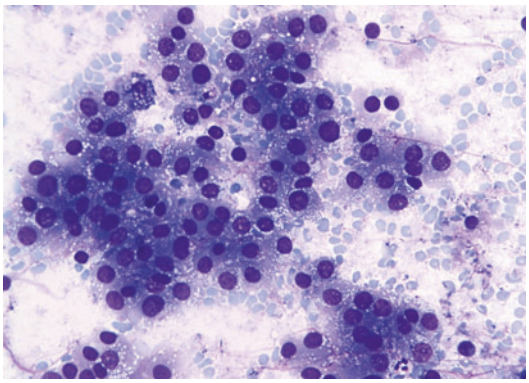
Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 4 Some of the granulosa cells have more abundant microvacuolated cytoplasm, consistent with the beginning of the luteinization (FNA. MGG ×1000)



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 6 Luteal cyst. Numerous sheets of large luteinized granulosa cells. Dispersed macrophages and fibroblasts may be encountered (FNA. MGG ×100)



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 5 Sheets and papillary clusters of the granulosa cells. Squamous cells as contaminants due to transvaginal aspiration (FNA. MGG ×100)



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 7 Luteinized granulosa cells with abundant cytoplasm which show numerous scattered microvacuoles. The nuclei are small, round, and eccentric (FNA. MGG ×400)

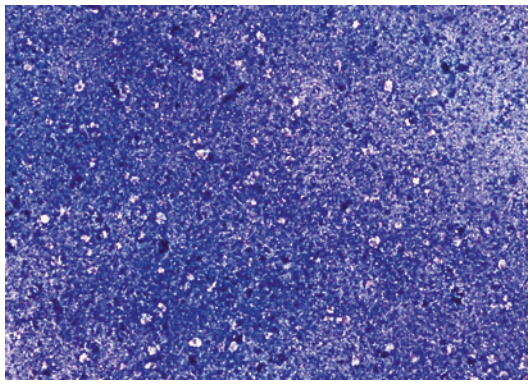
O

Definition

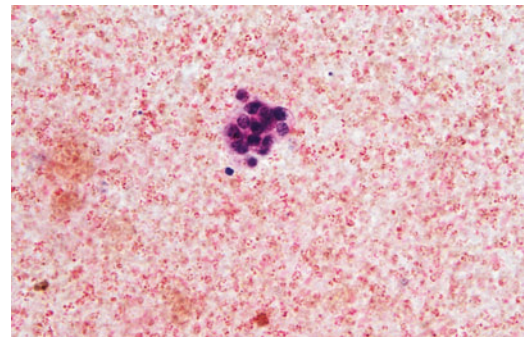
These are cystic transformation of the corpus luteum.

Cytologic Findings

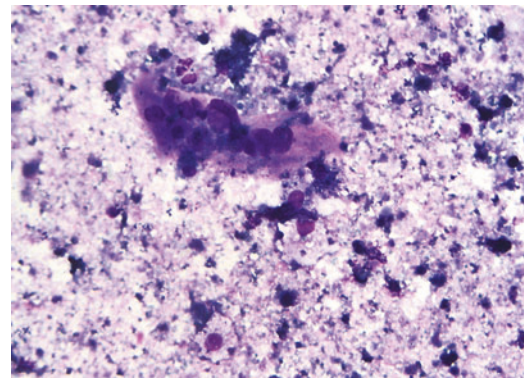
Fluid Aspiration (FNA) *Macroscopically* fluid is serosanguineous or clotted blood with a variable amount of organizing granulation tissue (Ivić et al. 1976; Bibbo et al. 2008). Blood not reabsorbed can coagulate, giving the corpus luteum cyst the appearance of a complex cyst on ultrasound. *Microscopically* the background is hemorrhagic, dense, granular with fibrin threads (Allias et al. 2000). Luteinized granulosa cells are large cells with abundant, oval, or polygonal, basophilic cytoplasm (Fig. 6) (Ivić et al. 1976). Their cytoplasms show numerous scattered microvacuoles. The nuclei are small, round, eccentric, often with uniform nucleoli (Fig. 7). Histiocytes and luteinizing cells were difficult to distinguish from one another due to vacuoles and pigment in both cell types. The accompanying smaller nonluteinizing granulosa cells provide a clue as to luteal, rather than histiocytic, origin of the large cells (Greenebaum et al. 1992). Macrophages with hematoidin pigment and numerous



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 8 Endometriosis of ovary. Typical dirty background composed of lysed red blood cells, cellular debris, and hemosiderin-laden macrophages (FNA. MGG ×100)



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 9 Endometriosis of ovary. A small cluster of endometrial cells in typical background (FNA. Papanicolaou ×400)



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 10 Endometriosis of ovary. Endometrial stromal cells in typical background (FNA. MGG ×400)

fibroblasts suggest a regressing corpus luteum (Manek and Mahovlić 2010).

Endometriotic Cyst

Synonyms

Chocolate cyst; Endometriomas; ► Endometriosis; Endometriotic cysts

Definition

Endometriotic cysts are cysts filled with blood arising from the ectopic endometrial glands and stroma. They occur most frequently in the ovary, but can be found almost anywhere in the body.

Cytologic Findings

Fluid Aspiration (FNA) *Macroscopically* fluid is hemorrhagic, dark brown (chocolate color) with abundant amounts of fresh and lysed erythrocytes and extracellular pigment. Chocolate-like cysts were not specific of endometriotic origin because hemorrhage may be seen in many types of cysts. *Microscopically* the diagnosis can be proposed because of a typical dirty background composed of lysed red blood cells, cellular debris, and hemosiderin-laden macrophages (Fig. 8), but it is not certified in the absence of epithelial cells (Allias et al. 2000). Endometrial epithelial cells come in bi- or tridimensional clusters, gland-like formations, or sheets of poorly preserved small cuboidal cells with round nuclei and scant cytoplasms (Fig. 9). They sometimes show nuclear molding and overlapping. Endometrial stromal cells may also be present (Fig. 10).

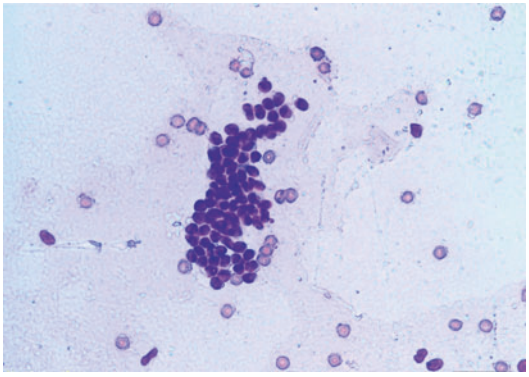
Simple Cyst

Synonyms

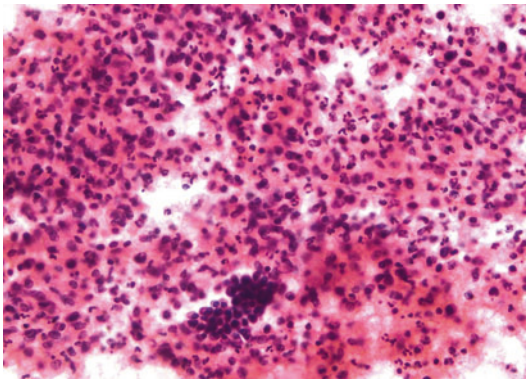
Germinal inclusion cyst; Simple cyst

Cytologic Findings

Fluid Aspiration (FNA) *Macroscopically* fluid is clear. *Microscopically* aspirations from simple cyst are generally paucicellular and the background is clean. The cells are mesothelial-like,



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 11 Simple cyst. Small sheet of low cuboidal cells (mesothelial-like) (FNA. MGG ×400)



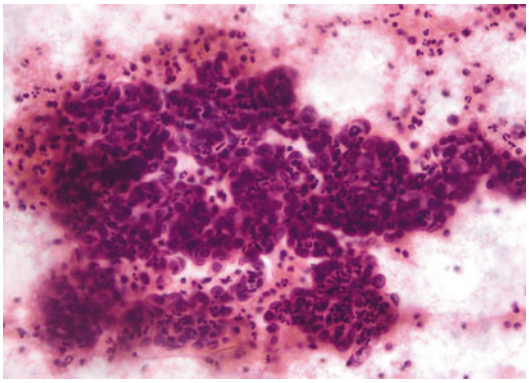
Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 12 Pelvic inflammatory disease. Numerous inflammatory cells with plasma cells aggregate (FNA. Papanicolaou ×400)

flat to cuboidal, with scanty cytoplasm and round, central nucleus (Fig. 11) (Allias et al. 2000).

Immunophenotype

Immunocytochemistry of nonneoplastic ovarian cysts.

Immunocytochemistry helps in distinguishing between hemorrhagic functional (corpus luteal or follicular), inhibin positivity, and endometriotic cysts, BerEP4/CA-125 positivity (Manek and Mahovlić 2010).



Ovarian Nonneoplastic Cysts, Cytological Findings, Fig. 13 Pelvic inflammatory disease. Numerous inflammatory cells phagocytized by tubal epithelial cells (FNA. Papanicolaou ×400)

Differential Diagnosis of Nonneoplastic Cysts of the Ovary

Serous ovarian or para-ovarian cysts characterized by the presence of ciliated columnar cells or ciliated fragments (ciliated bodies or tufts).

Pelvic inflammatory disease may be present as a cystic lesion, and the aspirates contain numerous inflammatory cells, lysed red blood cells, and sometimes tubal epithelial cells (Figs. 12 and 13).

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Pancreatic Ductal Adenocarcinoma, Cytological Findings

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Synonyms

Conventional pancreatic adenocarcinoma; Ductal adenocarcinoma; Ductal adenocarcinoma NOS; Pancreatic cancer; Usual ductal adenocarcinoma

Definition

Ductal adenocarcinoma of the pancreas is a malignant neoplasm of the exocrine pancreas that exhibits ductal differentiation.

Clinical Features

- **Incidence**
  Worldwide, pancreatic cancer is in the top ten leading causes of death from malignancy for both men and women, and ductal adenocarcinoma is the most common type of pancreatic cancer, constituting 85–90% of all pancreatic malignancies (Hruban et al. 2010).
- **Age**
  The age at diagnosis varies, but it is rarely found before the fifth decade of life.
- **Sex**
  There is a slight predilection for males, with a male to female ratio of 1.3:1.
- **Site**
  The majority (two thirds of cases) of ductal adenocarcinomas occur in the head of the pancreas, which are more often symptomatic, causing pain, jaundice, and weight loss. Distal tumors located in the body and tail of the pancreas comprise one third of cases, and are typically less symptomatic and thus larger at initial presentation (Hruban et al. 2007).
- **Treatment**
  Surgery is the mainstay of treatment of resectable tumors, with adjuvant therapy recommended for those patients who undergo surgery with curative intent. If the tumor is advanced and unresectable, palliative treatment options typically involve chemotherapy.
- **Outcome**
  The prognosis of patients with pancreatic ductal adenocarcinoma varies somewhat with tumor stage and grade. However, the overall survival is exceedingly poor, with only 4% of patients alive 5 years after diagnosis.

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Macroscopy

Ductal adenocarcinomas typically exhibit a gray-white, sclerotic gross appearance with ill-defined, infiltrative borders. Larger tumors may develop central necrosis that mimics a cystic neoplasm.

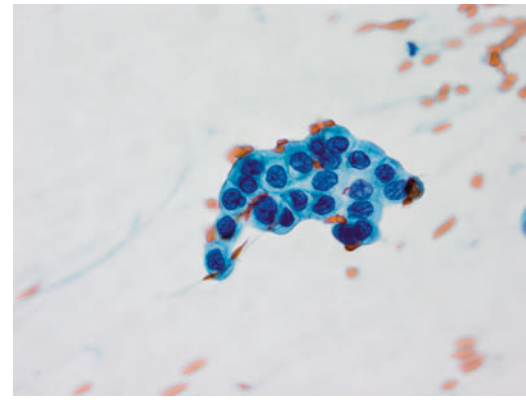
Microscopy (Cytologic Features)

Well-Differentiated Adenocarcinoma

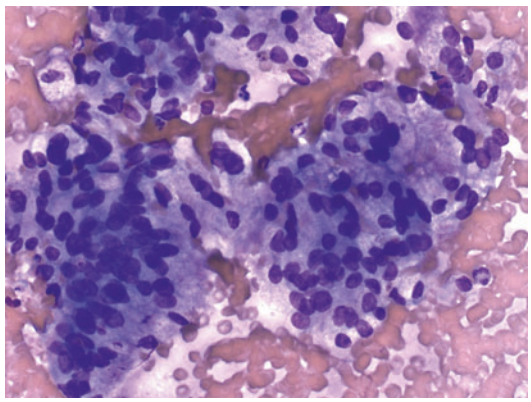
Fine needle aspiration biopsies of well-differentiated ductal adenocarcinomas typically yield cellular smears with tumor cells forming cohesive fragments of mildly atypical epithelium. The fragments may appear overly thick; instead of the normal, flat, evenly spaced "honeycomb" architecture found in benign ductal epithelium, the tumor cells exhibit nuclear crowding and overlap or are unevenly distributed in the sheet creating a "drunken honeycomb" pattern (Fig. 1). In comparison to higher grade tumors, the tumor cells exhibit minimal nuclear change, with mildly irregular nuclear contours, and occasional pyramidal or carrot-shaped nuclei (Fig. 2). The chromatin texture in well-differentiated adenocarcinoma is more likely to exhibit patchy chromatin clearing rather than coarseness (Fig. 3). Tumor cells may demonstrate mitotic activity, and the background may demonstrate granular debris indicative of necrosis, but these are present to a lesser extent than in higher grade tumors. Well-differentiated adenocarcinomas may be difficult to distinguish from reactive ductal epithelium.

Moderately Differentiated Adenocarcinoma

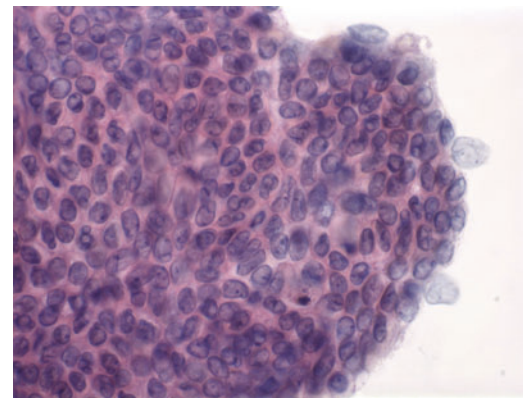
Moderately differentiated adenocarcinomas also typically yield cellular smears, with sheets of tumor cells; however, these tumors typically



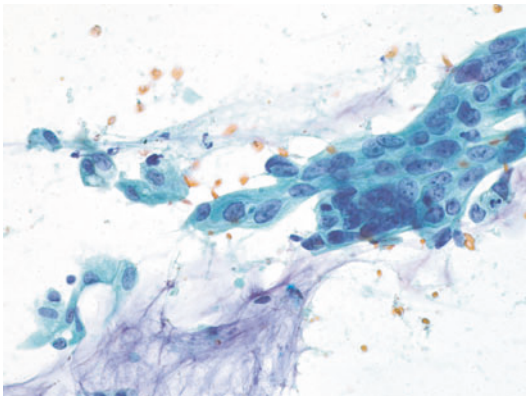
Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 2 While well-differentiated pancreatic adenocarcinomas often exhibit minimal nuclear atypia in comparison to higher grade tumors, scattered angulated nuclei that are pyramidal or carrot-like in shape can still be readily identified (Papanicolaou, ×600)



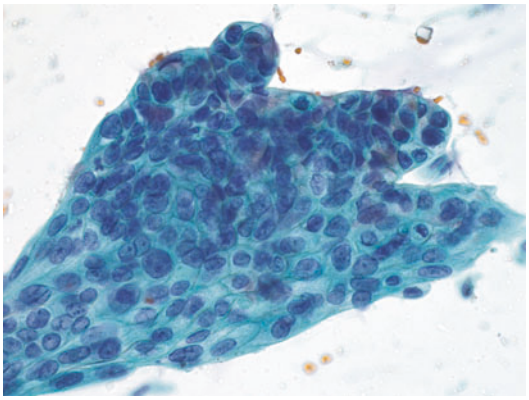
Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 1 The orderly "honeycomb" architecture in benign epithelium becomes disorganized in pancreatic adenocarcinoma, showing uneven spacing and forming a "drunken honeycomb" pattern (Diff-Quik, ×600)



Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 3 Well-differentiated pancreatic adenocarcinomas often exhibit nuclear pallor and parachromatin clearing than higher grade pancreatic adenocarcinomas (Papanicolaou, ×600)



Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 4 Moderately differentiated pancreatic adenocarcinomas exhibit more cellular dyscohesion than well-differentiated pancreatic adenocarcinomas, with single cells trailing off from larger fragments and visible in the background (Papanicolaou, ×600)

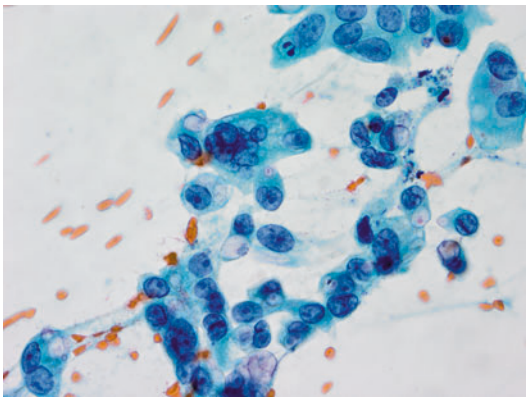


Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 5 Moderately differentiated pancreatic adenocarcinomas exhibit increased nuclear atypia in comparison to well-differentiated pancreatic adenocarcinomas. Nuclei are often very irregular in contour with readily identifiable notches, and exhibit increased coarseness to the chromatin (Papanicolaou, ×600)

exhibit a greater amount of dyscohesion. Single, atypical cells are scattered in the background and trail off from tumor fragments (Fig. 4). Intact fragments may exhibit a honeycomb architecture emphasized by cells with intracytoplasmic mucin, but many groups will appear more three-dimensional, with nuclear crowding and overlap. The nuclei exhibit more prominent nuclear membrane irregularity with convoluted and notched nuclear contours and increased coarseness of the chromatin in comparison to well-differentiated adenocarcinoma (Fig. 5). Prominent nucleoli may also be noted. Mitotic figures are more readily identifiable, and necrosis is more prominent. Moderately differentiated adenocarcinomas are more readily distinguished from a reactive process.

Poorly Differentiated Adenocarcinoma

Poorly differentiated adenocarcinomas demonstrate marked anisonucleosis and form loosely cohesive groups with many single cells. The aspirates typically exhibit a population of dyscohesive, highly atypical cells that are easily distinguished as a malignant process, with very high nuclear to cytoplasmic ratio, marked pleomorphism, and nuclei that demonstrate extreme nuclear contour irregularities and hyperchromasia (Fig. 6). Only focal glandular differentiation is identified which may be in the form of

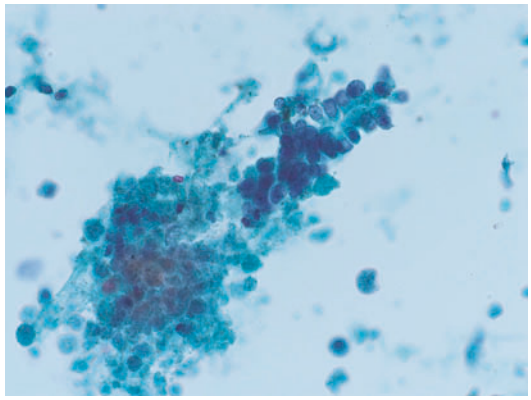


Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 6 Poorly differentiated pancreatic adenocarcinomas are composed of markedly pleomorphic cells in loose clusters and single cells (Papanicolaou, ×600)

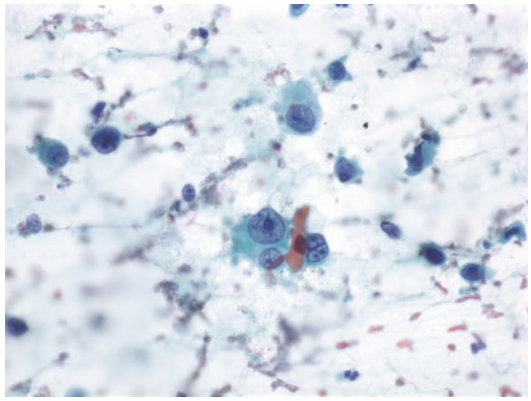
focal intracytoplasmic vacuoles. Mitotic figures are readily identifiable, and background necrosis is often prominent (Fig. 7).

Variants of Ductal Adenocarcinoma

There are many variants of the conventional ductal adenocarcinoma including adenosquamous carcinoma (Fig. 8), colloid (mucinous) carcinoma, hepatoid adenocarcinoma, medullary carcinoma, undifferentiated carcinoma with



Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 7 Unlike lower-grade tumors, poorly differentiated adenocarcinomas often contain prominent mitotic figures and background necrosis (Papanicolaou, ×600)

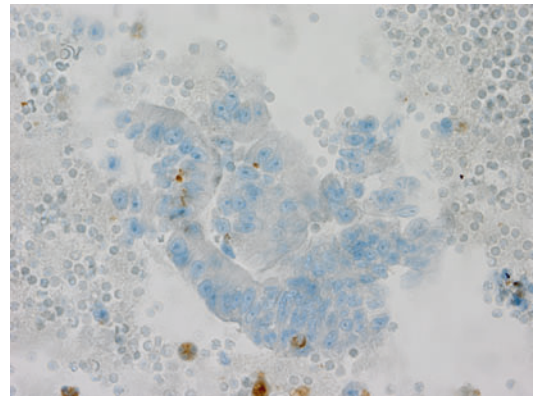


Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 8 Adenosquamous carcinomas exhibit features suggestive of both glandular and squamous differentiation, with some cells showing luminal borders and more delicate cytoplasm, and other cells exhibiting dense, glassy cytoplasm (Papanicolaou, ×400)

osteoclast-like giant cells, anaplastic carcinoma, and signet ring cell carcinomas.

Immunophenotype

Pancreatic ductal adenocarcinomas that are well differentiated may be difficult to distinguish from reactive epithelium. A recent study determined that a panel of stains including pVHL, maspin, S100P, and IMP-3 is most useful in distinguishing the two,



Pancreatic Ductal Adenocarcinoma, Cytological Findings, Fig. 9 SMAD4 is a nuclear immunohistochemical stain, and total loss of SMAD4 has been identified in close to 60% of pancreatic adenocarcinomas (SMAD-4 immunohistochemical stain, ×600)

both on surgical specimens as well as on fine needle aspiration biopsies. Loss of SMAD4, seen in about 60% of cases (Fig. 9), is also an indicator of pancreatic adenocarcinoma, but it is not entirely specific as it has been noted to be lost in other malignancies such as colorectal adenocarcinoma as well (Liu et al. 2012).

Molecular Features

Over 90% of pancreatic adenocarcinomas contain mutations in the oncogene *KRAS*, as well as inactivation of the tumor suppressor gene *CDKN2A*. Up to 85% of pancreatic cancers contain inactivating mutations in *TP53*, which is an important regulator of apoptosis, cell cycle progression, and DNA repair. Approximately 55% of pancreatic adenocarcinomas exhibit deletion of the *DPC4*/*SMAD4* gene, which is involved in the transforming growth factor beta (TGF-β) pathway that regulates cell proliferation and differentiation (Macgregor-Das and Iacobuzio-Donahue 2013; Vincent et al. 2011).

Differential Diagnosis

The main differential diagnosis for patients with ductal adenocarcinoma of the pancreas is chronic

pancreatitis. Pancreatic adenocarcinoma can be distinguished from these two benign entities by evaluating the epithelial architecture ("drunken honeycomb") and nuclear irregularities including nuclear crowding and overlap, nuclear membrane irregularity (shape and smoothness), and chromatin pallor (parachromatin clearing).

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Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings

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Synonyms

IPM adenoma; IPM carcinoma; invasive; IPMN with borderline carcinoma; IPMN with carcinoma in situ; IPMN with high-grade dysplasia; IPMN with intermediate-grade dysplasia; IPMN with invasive carcinoma; IPMN with low-grade dysplasia; IPMN with moderate dysplasia; Mucin-producing tumor; Mucinous duct ectasia; Papillary adenoma or carcinoma; Villous adenoma

Definition

The term "intraductal papillary mucinous neoplasm (IPMN)" is a broad category of mucin-producing ductal epithelial neoplasms that may involve the branch-ducts only, the main-duct only, or involve both. It is a neoplasm that produces a visible, macroscopic cystic, or solid lesion in contrast to similar microscopic lesions of the ductal system known as pancreatic intraepithelial neoplasia.

Clinical Features

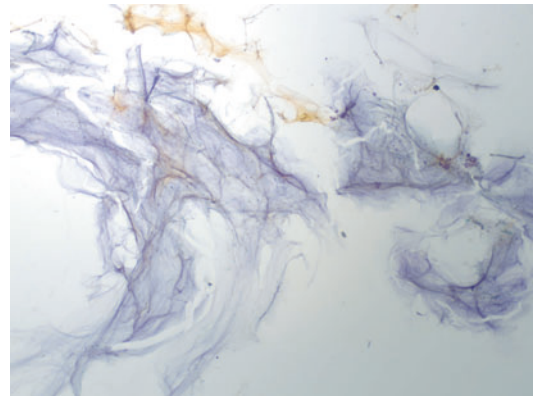
- **Incidence**
  Most IPMNs are asymptomatic, so the true incidence in the population is unknown. It is estimated that IPMNs represent about 2.8% of cysts detected by CT scan in the general population and over 8% of cysts in patients over 80 years of age.
- **Age**
  Adults ranging from third to ninth decade are affected by this neoplasm. The average age is 66 years for all neoplasms; patients with non-invasive neoplasms are about 3–5 years younger than patients with invasive IPMN.
- **Sex**
  The gender dominance varies by study, but overall, there appears to be a slight predominance in males.
- **Site**
  IPMNs can involve the whole pancreas or a portion of the gland. They are most common in the pancreatic head and uncinate process. Multiple branch-duct cysts occur in up to 40% of patients, and this finding on imaging is a clue to the diagnosis.
- **Treatment**
  IPMNs that involve the main pancreatic duct, whether main or combined type, are resected due to the high risk of malignancy. Branch-duct IPMNs can be managed conservatively,

especially in elderly patients with comorbid conditions. Resection is recommended if the lesion demonstrates high-risk features on imaging, or FNA confirms the presence of high-grade epithelial cells, resulting in a cytological interpretation of suspicious or positive for an IPMN with high-grade dysplasia or invasive carcinoma (Tanaka et al. 2012).

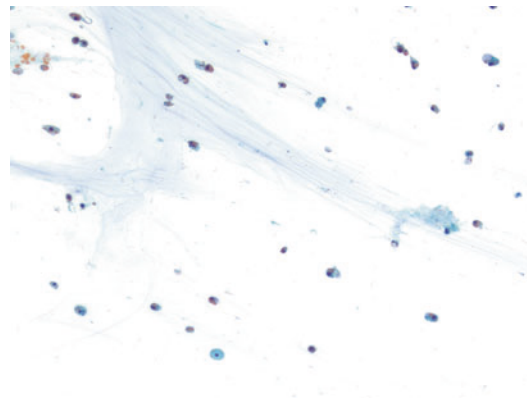
- **Outcome**
  The most important determinant of outcome is whether the IPMN is associated with an invasive carcinoma. The 5-year survival rate for IPMN with invasive carcinoma is between 27% and 60%, whereas 5-year survival for resected noninvasive IPMNs is close to 100%. Death from disease in patients with noninvasive IPMN is thought to be due to recurrence at the margin in a few, but more often due to metachronous multifocal disease of not only IPMN, but of conventional ductal adenocarcinoma as well.

Macroscopy

IPMNs are usually > 1.0 cm both for the diameter of the main pancreatic duct dilatation and the diameter of the cyst formed from the dilated branch-duct. Papillarity of the duct lining is variable as is the amount of grossly detectable mucin. The gross detection of thick, viscous mucin at the time of aspiration biopsy is typically a diagnostic feature of a mucinous cyst, but this is not a feature that distinguishes IPMN from mucinous cystic neoplasm.



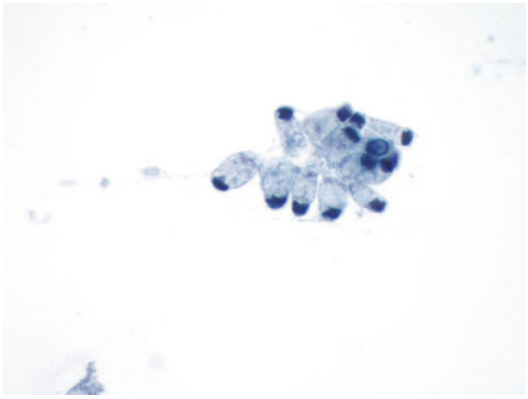
Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 1 Thick, colloid-like extracellular mucin without an epithelial component is sufficient cytological evidence to support the diagnosis of a mucinous cyst (Papanicolaou, 100×)



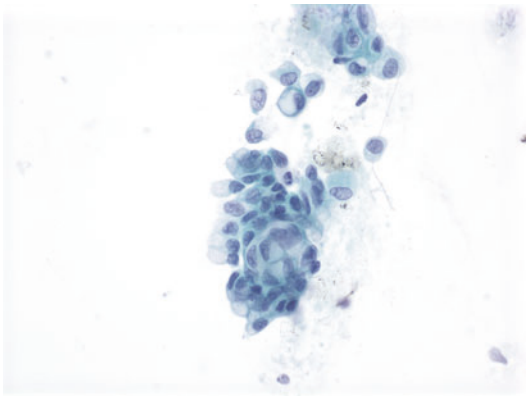
Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 2 Cellular debris within thin extracellular mucin supports the origin of the mucin from the cyst and not from the gastrointestinal tract during EUS-FNA (Papanicolaou, 100×)

Microscopy (Cytologic Features)

The quantity and quality of extracellular mucin and epithelium aspirated from an IPMN is typically dependent on the size and complexity of the cyst, and these two components are not necessarily directly related. For example, a cyst may produce thick, colloid-like extracellular mucin without an epithelial component in the sample, and this finding is sufficient to be diagnostic of a mucinous cyst (Fig. 1). Cellular debris within extracellular mucin supports the origin of the mucin from the cyst rather than the gastrointestinal tract during EUS-FNA (Fig. 2). If extracellular mucin is not visible or of uncertain origin, CEA analysis with a level at or above 192 ng/ml or detection of a *KRAS* mutation has been shown to be an accurate marker for a mucinous neoplasm (Cizginer et al. 2011; Khalid et al. 2009). Once the cyst is determined to be mucinous, then the epithelial cells needed to be classified as having either low-grade or high-grade morphology.



Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 3 IPMN with low-grade dysplasia; benign-appearing mucinous epithelium morphologically similar to contaminating gastric epithelium from a transgastric EUS-FNA (Papanicolaou, 600×)



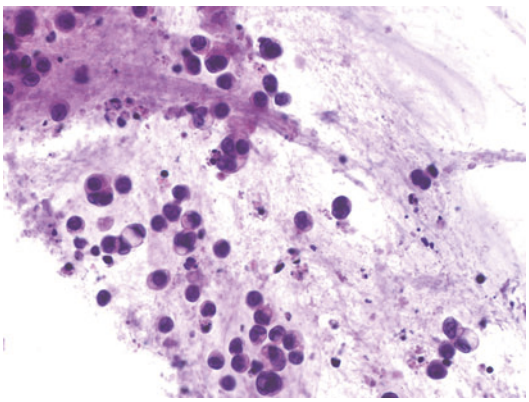
Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 4 IPMN with intermediate-grade dysplasia. Single cells and small clusters with mild-to-moderate atypia are best classified as low-grade morphology (Papanicolaou, 400×)

It can be very difficult to distinguish gastrointestinal intestinal contamination from low-grade dysplasia and high-grade dysplasia from invasive carcinoma, but generic classification into low or high-grade groups is sufficient for patient management (Pitman et al. 2010).

IPMN with low-grade dysplasia generally produce scantily cellular aspirates of benign-appearing mucinous epithelium that can be impossible to distinguish from gastric epithelium in transgastric EUS-FNAs (Fig. 3). Documentation in the cytology report on the absence of high-grade epithelial atypia may be sufficient for conservative patient management.

IPMN with intermediate-grade dysplasia produces epithelium that is difficult to accurately classify as low- or high-grade morphology. The cellular features of single cells and small clusters may appear more atypical in the cyst fluid than the correlating histological appearance (Fig. 4). Since the management of patients with intermediate-grade dysplasia in radiologically noncomplex branch-duct IPMN is not well defined, it is better to be more conservative and group mild-to-moderate epithelial atypia in the low-grade category.

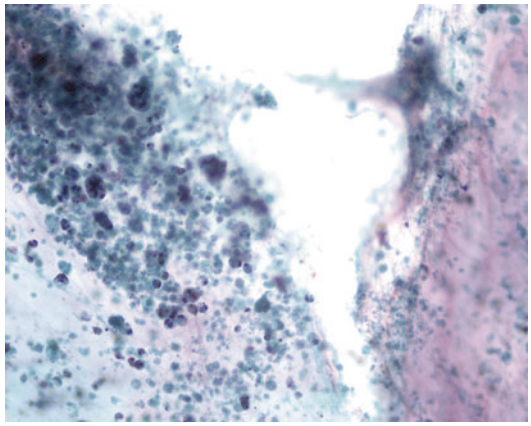
IPMN with high-grade dysplasia produces moderate to markedly atypical epithelial cells with nuclear crowding and membrane abnormalities, loss of polarity, nuclear elongation or



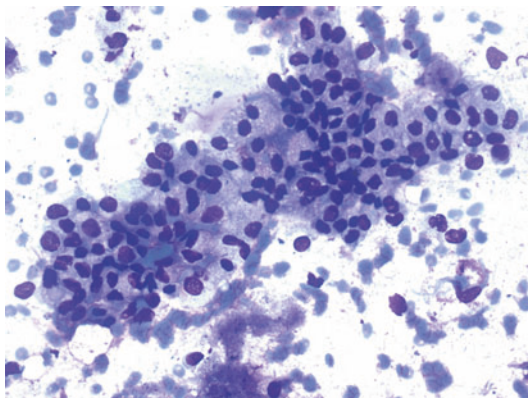
Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 5 IPMN with high-grade dysplasia produces moderate to markedly atypical epithelial cells with nuclear crowding, loss of polarity, nuclear elongation or rounding, hyperchromasia, and increased nuclear to cytoplasmic ratio (Hematoxylin and eosin, 400×)

rounding, hyperchromasia, and increased nuclear to cytoplasmic ratio (Fig. 4). Background cellular necrosis is usually minimal. Cells present in small, tight, bud-like clusters or singly with these same nuclear features and cytoplasm with or without mucin (Fig. 5).

IPMN with invasive carcinoma produces more abundant background necrosis than tumors with HGD (Fig. 6), and the epithelial cells meet the criteria for adenocarcinoma with cellular and



Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 6 IPMN with invasive carcinoma produces more abundant background necrosis than tumors with HGD (Papanicolaou, 200×)



Pancreatic Intraductal Papillary Mucinous Neoplasm, Cytological Findings, Fig. 7 IPMN with invasive carcinoma sheds epithelial cells that meet the criteria for adenocarcinoma with cellular and crowded groups of cells with open chromatin, irregular nuclear membranes and nucleoli and a drunken honeycomb pattern (Romanowsky stain, 600×)

crowded groups of cells with a drunken honeycomb pattern, and cells with open chromatin, irregular nuclear membranes and nucleoli and (Fig. 7).

Immunophenotype

There is often insufficient cyst fluid and cellular content to make an adequate cell block since most cysts that are triaged to FNA are usually smaller, less complex cysts that produce cyst fluid without a significant cellular component. The determination that the cyst is mucinous is most accurate with CEA analysis of fresh supernatant cyst fluid and the best determinate that the cyst is high-grade is with routine light microscopy. Since virtually all IPMNs have a high amylase level, testing the cyst fluid for amylase may be helpful in distinguishing IPMN from other pancreatic cysts that do not have high amylase levels such as serous cystadenoma and cystic neuroendocrine tumor. Amylase levels do not distinguish IPMN from mucinous cystic neoplasm (MCN). A cellblock with an epithelial component can be evaluated with CDX2 to distinguish duodenal epithelium from lesional epithelium. Expression of SMAD-4 is preserved in most noninvasive IPMNs, so a loss of SMAD-4 may indicate an invasive component.

Molecular Features

Greater than 96% of IPMNs have either a v-ki-ras2 Kirsten rat sarcoma (*KRAS*) or guanine nucleotide binding protein alpha stimulating, activity polypeptide 1(*GNAS*) mutation, and more than half harbor both mutations (Wu et al. 2011, #3896). *KRAS* mutations predominantly occur on codons 12 and 13; however, more than one mutant clone of *KRAS* may occur in a single IPMN cyst, and distinct IPMN cysts often have different *KRAS* mutations. *GNAS* mutations in PCF appear to occur only at codon 201, and this mutation seems to be specific to IPMNs and the invasive carcinomas arising from them. The detection of a *KRAS* mutation supports the presence of a neoplastic mucinous cyst, and the detection of a *GNAS* mutation distinguishes an IPMN from a MCN. Unfortunately, these molecular markers are present in all grades of dysplasia, so the detection of neither mutant gene distinguishes a low-grade from high-grade mucinous cyst. In addition, the absence of both markers does not exclude a mucinous neoplasm. In IPMN, the most frequent LOH occurs at chromosome 17q which involves the gene RNF43 (Wu et al. 2011), a gene associated with E3 ubiquitin ligase activity

(Sugiura et al. 2008). Some studies have suggested that the quality and quantity of DNA and order in which *KRAS* mutations and LOH occur can distinguish benign from malignant IPMN (Khalid et al. 2005).

Differential Diagnosis

The differential diagnosis of a pancreatic cyst includes both non-neoplastic and neoplastic primary cysts of the pancreas. The primary differential diagnosis of IPMN is MCN. On cytology alone, IPMN and MCN are not easily distinguished because the epithelium in the cyst fluid is not usually markedly papillary to support an IPMN and the subepithelial ovarian type stroma of an MCN is not usually appreciated on FNA. Cyst fluids without obvious extracellular mucin and that have a low CEA and absent *KRAS* mutation may represent a pseudocyst, a serous cystadenoma, a lymphoepithelial cyst, and solid pseudopapillary neoplasm. Secondarily cystic solid neoplasms such as neuroendocrine tumor should also be considered in the differential diagnosis.

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Pancreatic Lymphoepithelial Cyst, Cytological Findings

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Definition

Lymphoepithelial cysts (LECs) are extremely rare, non-neoplastic cysts of the pancreas that have a mature squamous cell lining and subepithelial lymphoid stroma (Adsay et al. 2002; Sakorafas et al. 2012).

Clinical Features

- **Incidence**
  LECs comprise less than 0.5% of all cystic pancreatic lesions and there are less than 100 cases of LECs reported (Adsay et al. 2002). Unlike LECs in other locations, LECs of the pancreas do not appear to be associated with HIV or autoimmune disorders (Adsay et al. 2002). It is hypothesized that these cysts may arise from epidermal inclusion cysts in pancreatic lymph nodes or branchial cleft cysts that have fused with pancreatic remnants during embryogenesis. Their true origin is unknown (Adsay et al. 2002; Sakorafas et al. 2012).
- **Age**
  LECs generally occur in the fifth to seventh decades of life (Sakorafas et al. 2012).
- **Sex**
  The majority of LECs occur in men, with a 4:1 ratio of men to women (Sakorafas et al. 2012).

- **Site**
  LECs show no site predilection within the pancreas but usually arise from just beneath the surface of the pancreas and protrude out into the surrounding soft tissue (Sakorafas et al. 2012).
- **Treatment**
  LECs are benign and require no treatment if LEC is diagnosed with certainty. Patients can simply be followed. If LECs become symptomatic, conservative surgical resection can be considered (Sakorafas et al. 2012).
- **Outcome**
  LECs are benign lesions (Sakorafas et al. 2012).

Macroscopy

LECs are usually solitary, and well circumscribed with a brown-yellow, grumous filling. They can be unilocular or multilocular (Adsay et al. 2002; Raval et al. 2010).

Microscopy (Cytologic Features)

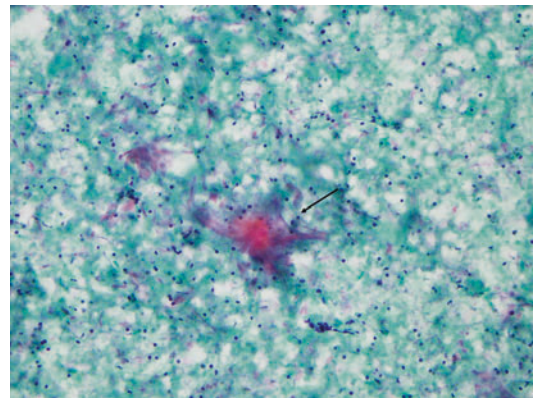
Cytology of LECs generally shows anucleated squamous cells, mature superficial squamous cells, and cholesterol clefts (Fig. 1). Lymphocytes and histiocytes are usually present but are highly variable in amount (Hruban et al. 2007).

Immunophenotype

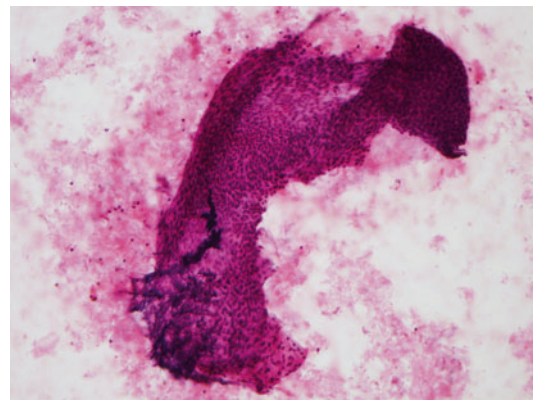
The squamous lining of the LECs is positive for CK7, p63, CEA, MUC1, and MUC4, and negative for MUC2, MUC5AC, and MUC6 (Raval et al. 2010). It is not necessary to perform these markers to diagnose LEC as the cytological features of LEC are distinctive (Fig. 2).

Molecular Features

Molecular testing has not been performed extensively on LECs. One study reports a single case of LEC as having no KRAS mutation (Ogura et al. 2012). Another series showed that three of nine



Pancreatic Lymphoepithelial Cyst, Cytological Findings, Fig. 1 Cluster of anucleate squamous cells (arrow) in a background of keratinaceous debris and lymphocytes. Papanicolaou stain, 40×



Pancreatic Lymphoepithelial Cyst, Cytological Findings, Fig. 2 Fragment of squamous lining from a LEC. H&E stain, 20×

LECs had levels of CEA greater than 450 ng/ml. LEC, therefore, may be a potential confounder of the greater than 192 ng/ml cut-off for mucinous neoplasms (Raval et al. 2010).

Differential Diagnosis

The differential includes other squamous lined cysts. Dermoid cysts often have other elements beside the squamous cells and keratin seen in LECs: sebaceous material, columnar cells, mesodermal elements, or hair. Pseudocysts lack the keratin debris and squamous cells found in LECs. Squamous cell carcinoma has greater

atypia than seen in LECs. Epidermoid cysts in intrapancreatic accessory spleens would be difficult to distinguish from LECs based on cyst fluid cytology alone but on surgical would show red pulp (Adsay et al. 2002; Hruban et al. 2007).

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Pancreatic Mucinous Cystic Neoplasm, Cytological Findings

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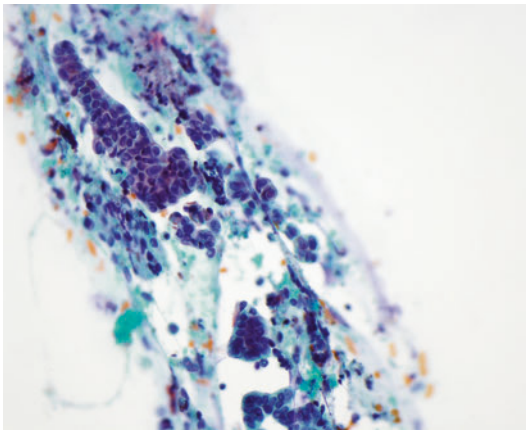
Synonyms

Cystic mucinous neoplasm; Mucinous cystadenoma; Mucinous cystic tumor



Pancreatic Mucinous Cystic Neoplasm, Cytological Findings, Fig. 1 Mucinous cystadenocarcinoma with mucinous and necrotic background. Sheets of crowded, malignant cells with high N/C ration, hyperchromatic nuclei and vesicular chromatin. Papanicolaou stain, 40×

Definition

Mucinous cystic neoplasm (MCN) is a mucin-producing, neoplasm of the pancreas with subepithelial ovarian-like stroma and no connection to the main pancreatic duct. MCNs have malignant potential and can transform into mucinous cystadenocarcinomas (MCAs, Fig. 1) (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

Clinical Features

Hamilton and Aaltonen (2010), Hruban et al. (2007), Pitman et al. (2010).

- **Prevalence**
  MCNs are rare: 1–2.6% of the general population will have a pancreatic cyst, and of those, 10% will be neoplastic and 5–10% of those will be an MCN.
- **Age**
  MCNs occur in the fifth to seventh decade of life, with a median of 54 years.
- **Sex**
  MCNs occur predominantly in women.

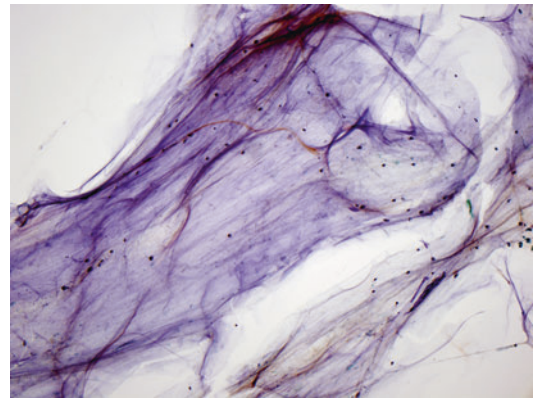
- **Site**
  Greater than 90% arise in the tail or body of the pancreas.
- **Treatment**
  Excision is the treatment of choice regardless of grade due to the generally young age of the patient, the eventual progression to malignancy, and the expense of life-long follow-up (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).
- **Outcome**
  The prognosis after resection of MCN is excellent if it is completely resected (100% cure with 0% recurrence). The prognosis becomes progressively poorer with aggressive features such as invasion, obstruction, rapid growth, and presence of malignant components (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

Macroscopy

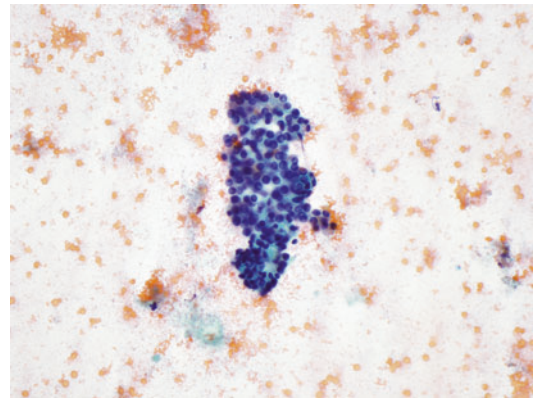
MCN is usually a solitary cyst with a thick fibrous wall, septations, and mucin production. Some may be multilocular and the quantity, and viscosity of the contents varies but is typically thick and difficult to aspirate (Hamilton and Aaltonen 2010; Hruban et al. 2007).

Microscopy

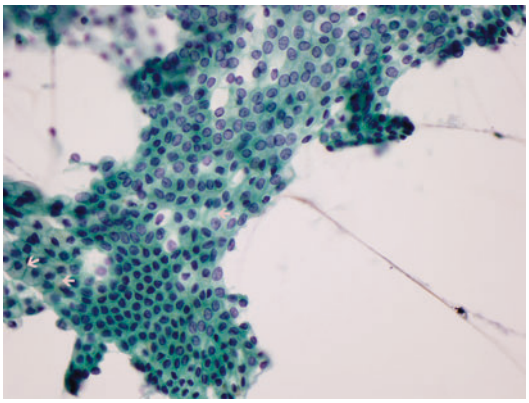
On cytologic examination of cyst fluid, the level of cellularity is typically scant (especially in lesions with less atypia) with background mucin. The level of mucin can also vary from thick sheets of undeniably pancreatic origin to thin wisps which are indistinguishable from gastric contamination (Fig. 2). MCN epithelium sometimes denudes and the cyst fluid may have debris. This can be mistaken for the debris of a pseudocyst (PCT) or necrosis (Fig. 3). MCN epithelium is usually columnar and can be arranged in sheets, pseudoacinar groups, papillary groups, palisading columns, or single cells (Fig. 4). Cells have well-defined redundant borders. MCN epithelium can show mild to severe atypia, including outright carcinoma in the setting of transformation to MCA. In the setting of MCA, the cyst fluid tends to be cellular with blood and necrosis. Cells will have frankly malignant features (high nuclear to cytoplasmic ratio, nuclear irregularities, and irregular chromatin). MCAs can also demonstrate a variety of architectures (sheets, acinar formations, papillary groups) or have a single-cell pattern with cytoplasmic vacuoles or signet cells. Mitoses or tumor ghost cells can be seen (Pitman



Pancreatic Mucinous Cystic Neoplasm, Cytological Findings, Fig. 2 Thick sheets of background mucin from MCN EUS-FNA. Papanicolaou stain, 40×



Pancreatic Mucinous Cystic Neoplasm, Cytological Findings, Fig. 3 MCN epithelium on dirty background of degraded blood with few inflammatory cells. Papanicolaou stain, 40×



Pancreatic Mucinous Cystic Neoplasm, Cytological Findings, Fig. 4 Mucinous epithelium in sheets, with well-defined cell borders and cytoplasmic mucin (*white arrows*). Papanicolaou stain, 40×

et al. 2010; Recine et al. 2004; Stelow et al. 2008; Obeso et al. 2009).

Immunophenotype

The epithelial cells in MCN are positive for CK7, 8, 18, 19, and EMA and are less reliably positive for CM20, CEA, DUPAN-2, and CA-19-9. The ovarian-like stroma is positive for SMA, alpha-inhibin, Melan-A, CD99, ER, and Bcl-2 (Pitman et al. 2010; Recine et al. 2004; Stelow et al. 2008; Obeso et al. 2009).

Molecular Features

Cyst fluid analysis can be especially helpful in the diagnosis of MCN. Carcinoembryonic antigen (CEA) should be greater than 192 ng/mL (Pitman et al. 2010) for a presumptive diagnosis of MCN, with a level of greater than 800 ng/mL being 98% specific for a mucinous neoplasm (MCN or intraductal papillary mucinous neoplasm (IPMN)), while cyst fluid amylase is generally low (less than 250 IU/mL). KRAS is commonly mutated in mucinous neoplasias and loss of heterozygosity may be present on cyst fluid DNA analysis (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

Differential Diagnosis

Differential diagnosis for MCN includes pancreatic IPMN, benign gastrointestinal (GI) epithelium, serous cystadenoma (SCA), and PCT.

The distinction between MCN and IPMN can be quite difficult as both may produce copious to scant mucin, and may shed columnar, mucinous, epithelial cells in strips which show diffuse mucin staining. In many cases, cytology may be signed out as "mucinous neoplasia," as distinguishing the two may be impossible. MCNs and IPMNs can also be confused for gastrointestinal contamination. GI mucin-producing cells tend to have concentrated, apical mucin cups (foveolar epithelium or goblet cells) as opposed to the more diffuse, cytoplasmic mucin content of MCN.

When separating MCN from other non-mucinous cysts, the quality and quantity of the cyst fluid is important. Thick, viscous, mucin-rich fluid virtually excludes PCT and SCA which have thinner contents and, in the case of SCA, may contain very little fluid at all.

In denuded MCN, the fluid can closely resemble the dirty aspirate of a PCT. PCTs will present with a dirty background filled with proteinaceous debris, pigment-laden macrophages, and other inflammatory cells. There should be no epithelium in PCTs. GI mucin and epithelial contamination can cloud the diagnosis, but as discussed previously, GI epithelium should appear morphologically distinct from MCN and lacks copious background mucin.

SCAs also may contain only scant amounts of thin fluid and are devoid of mucin. The epithelium, if any, seen in cytology specimens of SCA should be bland, cuboidal, and present in small strips as opposed to the columnar, mucinous cells of MCN (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

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Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings

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Synonyms

Gelatinous carcinoma; Pure mucinous carcinoma

Definition

Colloid (mucinous noncystic) carcinoma is a variant of pancreatic ductal adenocarcinoma characterized by fragments of mucinous epithelium suspended in abundant, well-defined pools of extracellular mucin; these tumors are morphologically similar to the colloid carcinomas of other tissues such as the breast and colon Adsay et al. 2001. The colloid component accounts for at least 50% of the tumor according to the World Health Organization (Kloppel et al. 2000); according to the Armed Forces Institute of Pathology, the colloid component accounts for at least 80% of the tumor (Hruban et al. 2007). Most cases of pancreatic colloid carcinoma arise in association with a low-grade, in situ papillary process such as an intraductal papillary mucinous neoplasm (IPMN) (Adsay et al. 2003).

Clinical Features

- **Incidence**
  Colloid carcinomas represent approximately 2% of all malignancies of the exocrine pancreas (Longnecker 2013).
- **Age**
  Patients with colloid carcinoma present in their sixth to seventh decades, similar to conventional pancreatic ductal adenocarcinoma (Adsay et al. 2001).
- **Sex**
  Colloid carcinoma shows no gender predilection (Adsay et al. 2001).
- **Site**
  Most cases of colloid carcinoma have been found in the pancreatic head, but cases of colloid carcinoma in the pancreatic body and tail have also been reported (Adsay et al. 2001; Liszka et al. 2008).
- **Treatment**
  Treatment options for patients with colloid carcinoma are similar to those with invasive IPMN and include pancreaticoduodenectomy, distal pancreatectomy, or total pancreatectomy depending on the location of the tumor (Liszka et al. 2008).
- **Outcome**
  It is currently unclear whether colloid differentiation is an independent predictor of patient survival. In a series of 17 cases reported by Adsay et al., colloid carcinomas (defined as >80% of the neoplasm showing colloid differentiation) appeared to have a better prognosis compared to conventional pancreatic ductal adenocarcinoma, with 5-year survival rates of 57% and 12% for patients with colloid carcinoma and conventional pancreatic ductal adenocarcinoma, respectively (Adsay et al. 2001). In contrast, Seidel et al. reported that patients with pancreatic colloid carcinoma (defined as tumors with at least 50% of the neoplasm showing colloid differentiation) demonstrated

a 5-year survival rate of 13% (compared to 21% for patients with conventional pancreatic ductal adenocarcinoma) (Seidel et al. 2002).

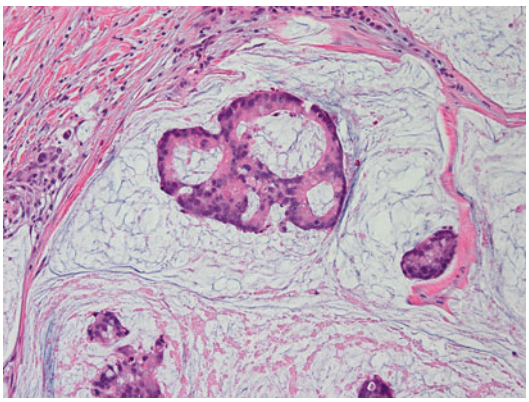
Macroscopy

Compared to conventional pancreatic ductal adenocarcinomas, colloid carcinomas tend to be larger with a yellow-white, glistening, gelatinous cut surface. Cystic spaces filled with mucoid material may be evident, particularly if the colloid carcinoma is associated with an IPMN or mucinous cystic neoplasm (Adsay et al. 2001; Centeno and Pitman 1999; Liszka et al. 2008).

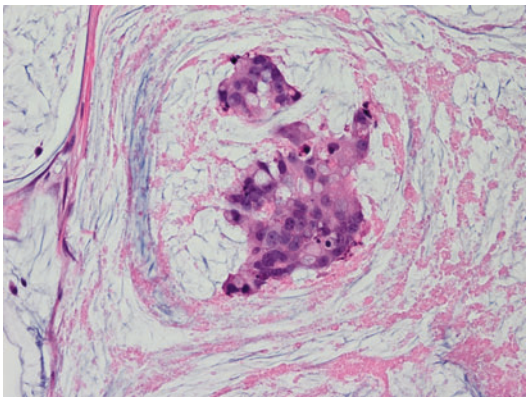
Microscopy

Histologic sections of colloid carcinoma show well-differentiated mucinous epithelium arranged in cords, trabeculae, irregularly shaped glands, and cribriform clusters suspended within abundant pools of mucin (Figs. 1–2). Partial lining of the stromal mucin pools by tumor epithelium can be seen. Individually dispersed signet ring cells may also be present.

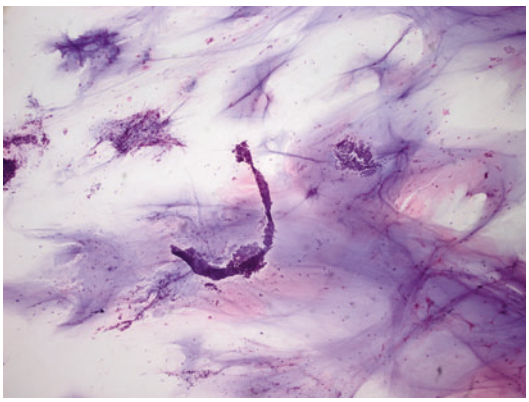
Fine-needle aspiration of mucinous neoplasms yields viscous, colloid-like fluid that is difficult to aspirate into and express from the needle (Pitman and Deshpande 2007). Aspirate smears consist of variably atypical cuboidal to columnar cells admixed with dense mucoid material (Figs. 3–6). Cells are often arranged in three-dimensional clusters (Figs. 4 and 5), sheets (Figs. 6 and 7), and/or dispersed as single cells. Intracytoplasmic mucin vacuoles may be present (Fig. 4). Nuclei are typically bland, eccentrically positioned, and show variably prominent nucleoli (Figs. 4–7).



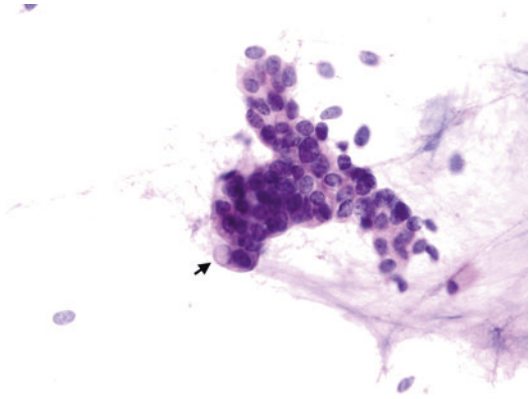
Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 1 H&E-stained section from a distal pancreatectomy specimen shows neoplastic mucinous epithelium suspended in well-defined pools of stromal mucin. This case of colloid carcinoma was associated with intestinal-type intraductal papillary mucinous neoplasm (H&E stain)



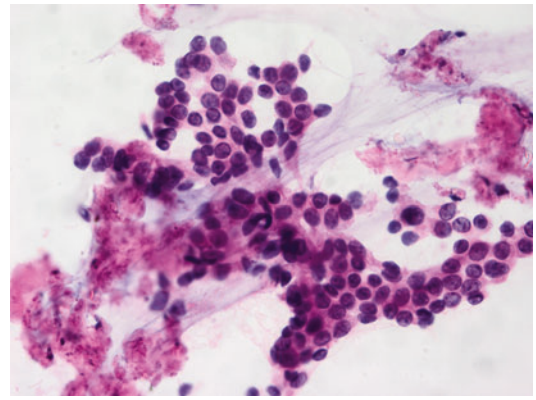
Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 2 H&E-stained section highlighting an irregular fragment of neoplastic mucinous epithelium floating within stromal mucin (H&E stain)



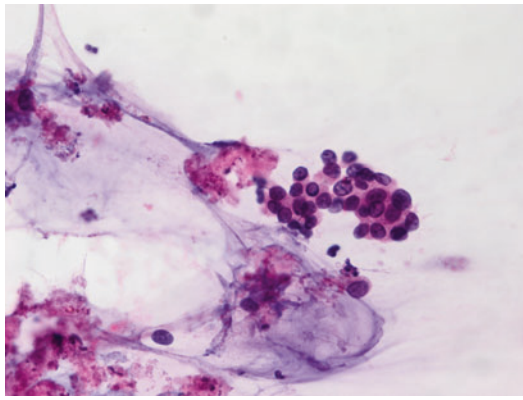
Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 3 Low-magnification image of an H&E-stained aspirate smear showing sheets of epithelial cells in a background of abundant extracellular mucin (H&E stain)



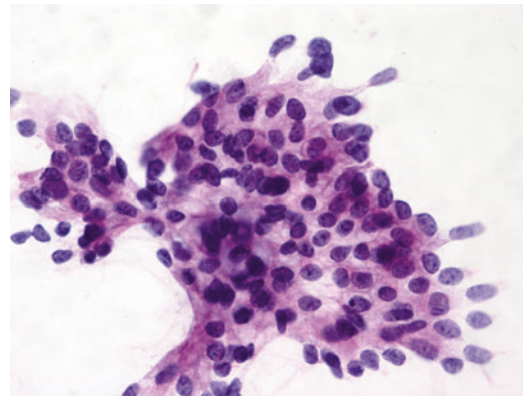
Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 4 H&E-stained aspirate smear showing a cluster of atypical epithelial cells with overlapping nuclei. An intracytoplasmic mucin vacuole (*arrow*) and abundant extracellular mucin are seen (H&E stain)



Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 6 High-magnification image of an H&E-stained aspirate smear showing atypical epithelial cells arranged in irregular sheets. Mucin and necrotic debris are present in the background



Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 5 High-magnification image of an H&E-stained aspirate smear showing a cluster of atypical epithelial cells with ovoid, partially overlapping, eccentric nuclei. Dense extracellular mucin and necrotic debris are present in the background (H&E stain)



Pancreatic Mucinous, Noncystic Adenocarcinoma (Colloid Carcinoma), Cytological Findings, Fig. 7 High-magnification image of an H&E-stained aspirate smear shows atypical epithelial cells with ovoid, eccentrically placed nuclei, slightly stippled chromatin, and small nucleoli. The cells have a moderate amount of cytoplasm

Immunophenotype

Pancreatic colloid carcinomas are typically positive for markers of intestinal differentiation such as CDX2 and MUC2 (Adsay et al. 2003, 2001).

Molecular Features

Pancreatic colloid carcinomas harbor *KRAS* and *P53* mutations similar to conventional pancreatic adenocarcinomas, but at a lower frequency (Adsay et al. 2001). Although loss of the

SMAD4/DPC4 gene product is seen in approximately half of pancreatic ductal adenocarcinomas, *SMAD4/DPC4* expression appears to be preserved in all cases of pancreatic colloid carcinoma reported to date.

Differential Diagnosis

Based on the presence of mucinous epithelium and abundant mucin, the cytologic differential diagnosis of pancreatic colloid carcinoma includes IPMN, mucinous cystic neoplasm, and conventional pancreatic ductal adenocarcinoma. Abundant mucin can also be seen in nonneoplastic entities such as mucoceles. The presence of atypical epithelial cells helps exclude nonneoplastic entities such as mucoceles. Correlation with imaging studies may be helpful to exclude IPMN and mucinous cystic neoplasms, and surgical resection may be necessary for definitive classification of the neoplasm.

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Pancreatic Neuroendocrine Tumor, Cytological Findings

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Synonyms

Carcinoid tumors; Gastrinomas; Glucagonomas; Insulinomas; Islet cell carcinoma; Islet cell tumors; Pancreatic endocrine tumor; Somatostatinomas; VIPomas

Definition

Pancreatic neuroendocrine tumors (PanNETs) are tumors with neuroendocrine differentiation that may be functioning (tumors which produce a hormonal syndrome) or nonfunctioning tumors (tumors which do not produce a hormonal syndrome). Nonfunctioning tumors can still express hormones but do not produce a clinical syndrome. The majority of PanNETs are non functional (Hruban et al. 2007).

Clinical Features

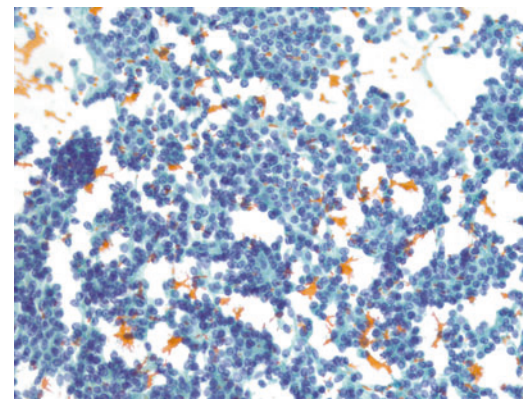
- **Incidence**
  PanNETs are rare, occurring in 1 out of 100, 000 people. Incidence at autopsy is 1.5% (Mansour and Chen 2004).
- **Age**
  In general, PanNETs occur in adults in their fifth decade. VIPomas occur in adults between 30 and 50 years old and in children between 2 and 4 years old (Oberg 2010; Mansour and Chen 2004).
- **Sex**
  Insulinoma and VIPomas have a slight predilection for women and gastrinomas for men (Hruban et al. 2007; Mansour and Chen 2004). Glucagonomas, somatostatinomas, and nonfunctioning PanNETs show no gender preference (Oberg 2010; Mansour and Chen 2004).
- **Site**
  Insulinomas can appear anywhere in the pancreas. Gastrinomas occur in the head and neck of the pancreas. Glucagonomas are found in the body and tail of the pancreas. Somatostatinomas are located mainly in the head of the pancreas and VIPomas generally in the tail of the pancreas (Mansour and Chen 2004). Nonfunctioning PanNETs occur in the head of the pancreas (Oberg 2010).
- **Treatment**
  In patients without metastatic disease, complete surgical resection is the treatment of choice. Even in patients with metastatic disease (by definition, malignant PanNETs), aggressive surgical debulking can be beneficial. Radiofrequency ablation, cryoablation, and hepatic artery embolization can be used to target hepatic metastases in addition to surgical resection. Liver transplantation may be considered. Chemotherapy, such as streptozocin and doxorubicin, may be used for neoadjuvant therapy. Peptide receptor radionucleotide therapy may be used in tumors with high somatostatin receptor expression (Mansour and Chen 2004).
- **Outcome**
  In patients with tumors limited to the pancreas, survival is 61% after 5 years. In patients with distant metastases, survival is 15% after 5 years. Survival is significantly better than that of pancreatic adenocarcinoma (American Cancer Society 2012).

Macroscopy

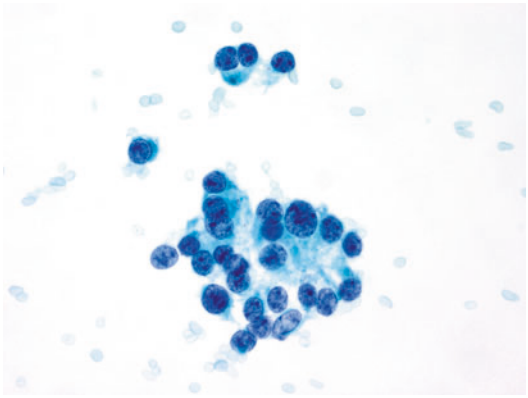
PanNETs are well-circumscribed, fleshy nodules, sometimes with central necrosis. Cystic PanNETs are not uncommon and can be confused with primary pancreatic cysts (Yoon et al. 2007).

Microscopy (Cytologic Features)

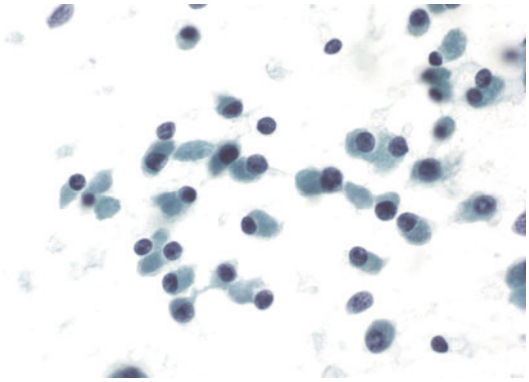
Most PanNETs are well-differentiated. Smears are usually very cellular with discohesive, single cells that produce a solid-cellular smear pattern (Fig. 1). Cells sometimes form rosettes or sheets (Fig. 2). Fibrovascular stroma can be seen with loosely attached tumor cells (Fig. 3). Nuclei are round to ovoid with salt and pepper chromatin and small nucleoli. Cells can be bi- or multinucleated. Cytoplasm is basophilic, wispy, and poorly defined. Most cells are plasmacytoid with



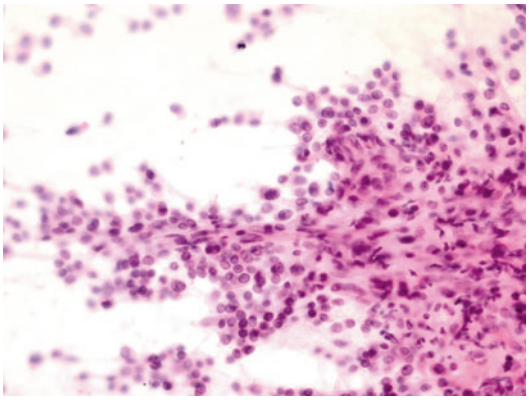
Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 1 Solid-cellular smear pattern of PanNET. Note that occasional rosettes are seen. Papanicolaou stain, 20×



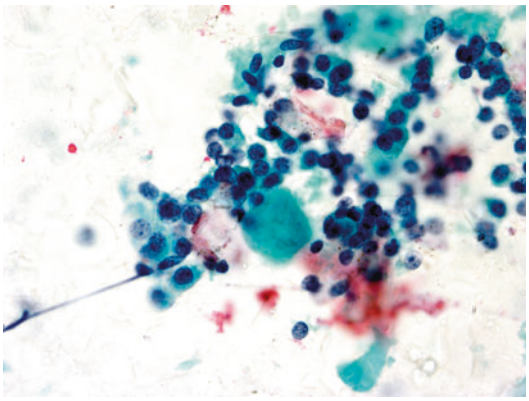
Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 2 High power view of a PanNET showing a rosette, speckled chromatin, nucleoli, and pleomorphism. Papanicolaou stain, 60×



Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 4 Plasmacytoid cells in a PanNET. Note the eccentric nuclei and abundant, dense, well-defined cytoplasm. Papanicolaou stain, 60×

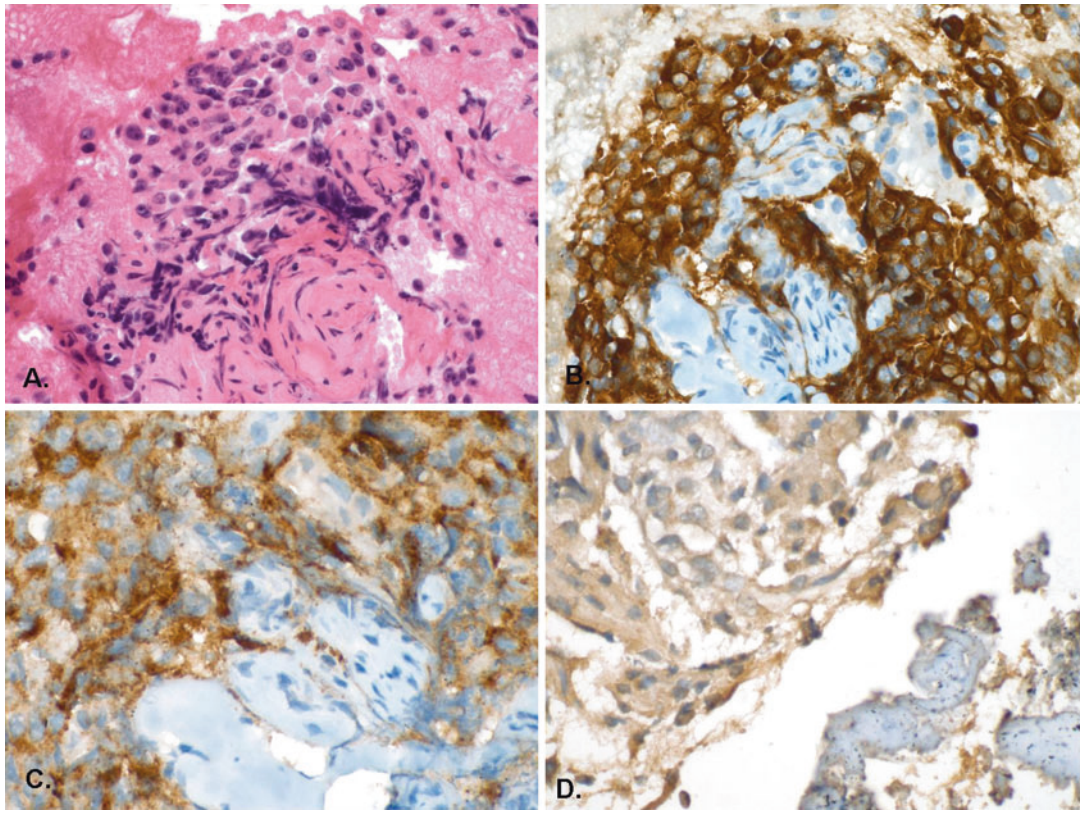


Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 3 Fibrovascular stroma of PanNET. H&E stain, 40×

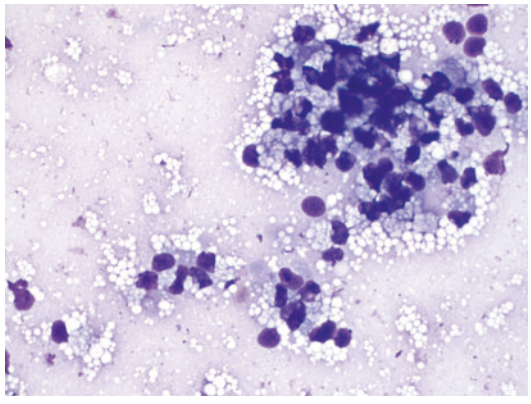


Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 5 Amyloid-like stroma in a PanNET. The smear showed abundant nodules of glassy, acellular, dense stroma. Papanicolaou stain, 60×

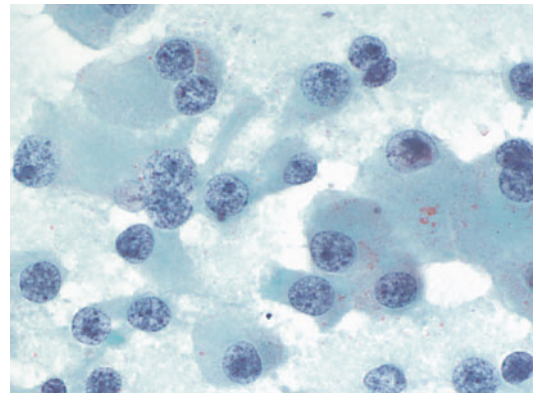
eccentric nuclei and dense, well-defined cytoplasm (Fig. 4). Pleomorphism and mitoses may be present but do not connote a more aggressive tumor. Psammoma bodies are occasionally seen. Amyloid or amyloid-like stroma may be present in insulin-secreting tumors (Fig. 5). Some PanNETs can have cystic changes, evidenced by a background of macrophages and debris (Fig. 9). Cyst fluid from cystic PanNETs has low CEA levels (median 1.1 ng/ml) compared to mucinous cysts of the pancreas (Yoon et al. 2013). Poorly differentiated PanNETs can be either small or large cell carcinomas. Small cell carcinomas show numerous mitotic figures (>10 mitoses per 10 high power fields) and tumor necrosis but otherwise look like a small cell carcinoma from the lung (Fig. 10). Large cell carcinomas have large pleomorphic nuclei, numerous mitotic figures, and necrosis. Variants of PanNETs include lipid-rich and oncocytic variants. Lipid-rich variants show vacuolated cytoplasm (Fig. 7).



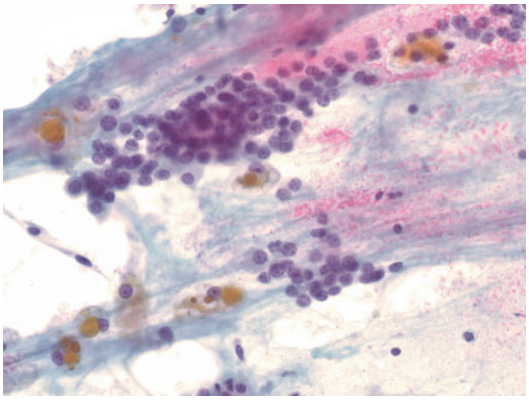
Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 6 Cell block of a PanNET. (**a**) H&E stain of the cell block. (**b**) Positive synaptophysin immunohistochemical stain. (**c**) Positive chromogranin immunohistochemical stain. (**d**) Negative trypsin immunohistochemical stain in a high background



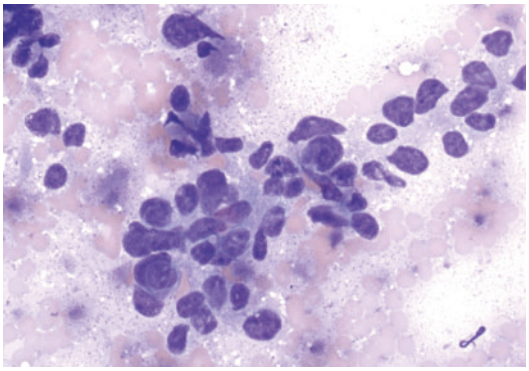
Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 7 Clear cell variant of PanNET with abundant vacuolated cytoplasm. Diff-Quik stain, 60×



Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 8 Oncocytic variant of PanNET with dense and granular cytoplasm. Papanicolaou stain, 60×



Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 9 PanNET with cystic changes. Note the hemosiderin-laden macrophages and acellular debris. Extracellilar mucin is gastrointestinal contamination. Papanicolaou stain, 60×



Pancreatic Neuroendocrine Tumor, Cytological Findings, Fig. 10 Small cell carcinoma with prominent nuclear molding. Diff-Quik stain, 60×

Oncocytic variants have oncocytic cells with dense, granular cytoplasm (Hruban et al. 2007) (Fig. 8).

Immunophenotype

PanNETS are positive for chromogranin, synaptophysin, CD56, and have variable positivity for peptide hormones depending on the tumor type. PanNETs are negative for trypsin (Hruban et al. 2007) (Fig. 6).

Molecular Features

Ninety percent of PanNETs occur sporadically. PanNETs can be associated with multiple endocrine neoplasia type 1 (MEN1) syndrome and von Hippel-Lindau (VHL) disease and rarely with neurofibromatosis 1 and tuberous sclerosis complex (TSC); 44% of sporadic PanNETs have mutations in MEN1, and 8.8% of sporadic PanNETS have mutations in TSC2. VHL mutations rarely occur sporadically. 11q loss is the most common abnormality in nonfunctioning PanNETs and benign insulinomas, 6q loss in malignant insulinomas, and 3p loss in gastrinomas. 17q gain is the most common finding in nonfunctioning PanNETs and malignant insulinomas, 9p gain in gastrinomas, and 9q gain in benign insulinomas. Alterations in expression of Ras, Src kinases, BCL2, cyclin D1, c-MYC, MDM2, MDM4, WIP1, CDKN2A, members of the PI3K/AKT/mTOR pathway, and various growth factors and receptors have also been reported in PanNETs. These genetic alterations may provide future opportunities for targeted therapy of PanNETs (Capurso et al. 2012).

Differential Diagnosis

Endocrine cell proliferation (endocrine cell hyperplasia or nesidioblastosis) shows a mix of endocrine and exocrine cells and greater cohesiveness than PanNETs. Endocrine cell proliferations also do not form a well-defined, well-circumscribed mass like PanNETs. Acinar cell carcinoma (ACC) on smear has bland acini in loosely cohesive groups with naked nuclei in the background. ACC are positive for trypsin and have PAS-/D-positive cytoplasmic granules. Solid-pseudopapillary neoplasm (SPN) shows branching, papillary clusters with fibrovascular cores and myxoid stroma. SPN is vimentin-positive and trypsin-negative. PAS/D-positive cytoplasmic hyaline globules are found in SPN. Adenocarcinoma has more cohesive clusters, prominent nucleoli, and abnormal chromatin distribution. Lymphoma has neither as much cytoplasm as PanNET nor the occasional cohesive

clusters seen in PanNET. Other plasmacytoid lesions (plasmacytomas, malignant melanoma, rhabdoid malignancies) may be distinguished with immunohistochemistry (Hruban et al. 2007).

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Pancreatic Pseudocyst, Cytological Findings

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Synonyms

Inflammatory pseudocyst

Definition

Pancreatic pseudocysts (PCTs) are cystic spaces which arise due to disruption of pancreatic parenchyma and ducts and the resulting formation of enzyme-rich, fluid-filled spaces without a true epithelial lining (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

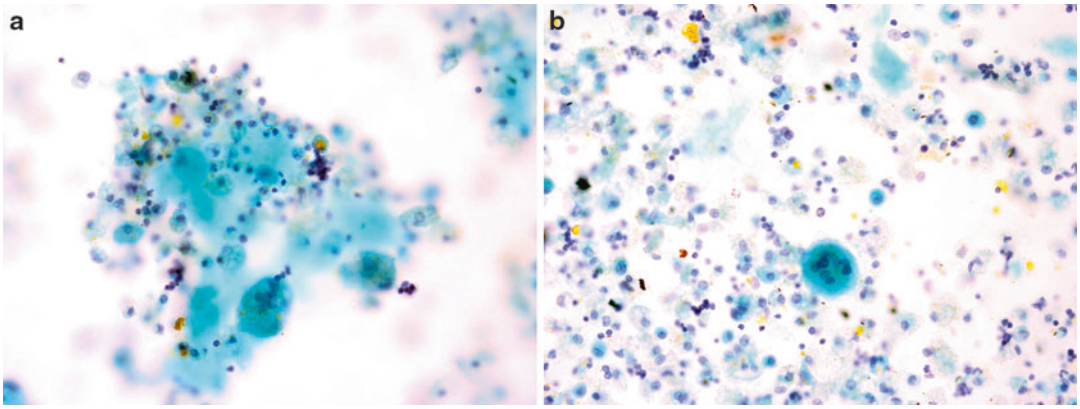
Clinical Features

Hamilton and Aaltonen (2010), Hruban et al. (2007), Pitman et al. (2010).

- **Incidence**
  PCTs have an incidence 0.5–1 per 100,000 adults per year or 1.6–4.5%.
- **Age**
  Age of occurrence is highly variable but strongly associated with acute and chronic pancreatitis.
- **Sex**
  PCTs predominantly occur in males.
- **Site**
  The cysts tend to be distributed throughout the pancreas, with no predilection for a certain region.
- **Treatment**
  PCTs are treated with watchful waiting unless symptomatic or if they show suspicious radiological features such as solid areas, obstruction of pancreatic ducts, or rapid increase in size. In such cases, they are treated by excision or endoscopic drainage.
- **Outcome**
  Outcome of drainage or excision is excellent, and there is no risk of malignant transformation (although coincidental as well as secondary coexistence with malignant neoplasms has been observed).

Macroscopy

PCTs typically have thick fibrous walls with irregular contours. The cyst fluid can be hemorrhagic or turbid and thin with debris, sometimes visible



Pancreatic Pseudocyst, Cytological Findings, Fig. 1 (**a** and **b**). Hemosiderin-laden macrophages, giant cells, inflammation, yellow extracellular pigment, and cyst debris in a PCT cyst fluid. Papanicolaou stain, 40×

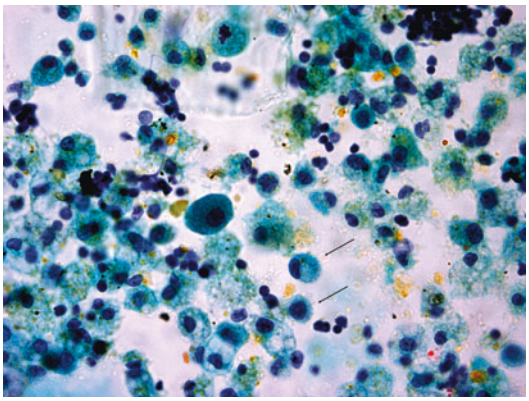
on radiographic imaging. If infection is present, thick, purulent inflammatory material can be present (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

Microscopy

Because there is no epithelial lining, examination of the cyst fluid is the principal means of analysis. The background is composed of cellular and proteinaceous debris and additionally may contain blood, necrotic fat, yellow extracellular crystalline pigment, fibroblasts, neutrophils, or histiocytes, often with ingested hemosiderin (Fig. 1a and b). Alternatively, the cyst fluid may be relatively clear and contain small numbers of histiocytes. In a true pseudocyst, epithelial cells should not be present apart from pancreatic and gastrointestinal (GI) contamination. Epithelioid histiocytes can sometimes be mistaken for mucinous epithelium (Fig. 2). Atypical epithelial cells are sometimes noted and, if seen, should raise the observer's suspicion of underlying malignancy (Pitman et al. 2010; Obeso et al. 2009; Stelow et al. 2008; Recine et al. 2004)

Immunophenotype

Because the pseudocyst is a structural result of ruptured ducts and has no characteristic cellular component, immunohistochemistry is of limited utility in diagnosis.



Pancreatic Pseudocyst, Cytological Findings, Fig. 2 Macrophages (*arrows*) can sometimes contain large vacuoles and appear more epithelioid. These can be mistaken for mucinous epithelial cells. Papanicolaou stain, 60×

Molecular Features

The cyst fluid should have carcinoembryonic antigen (CEA) of less than 192 ng/mL (Pitman et al. 2010). Cyst fluid amylase should be greater than 250 IU/mL and is typically in the thousands. Because pseudocysts are not neoplastic and are purely the result of mechanical obstruction, KRAS should be wild type and loss of

heterozygosity should be absent (Hruban et al. 2007; Pitman et al. 2010).

Differential Diagnosis

The differential diagnosis for pseudocyst includes serous cystadenoma (SCA) and mucinous neoplastic cysts (mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN)).

The principal entity in the differential diagnosis of pancreatic pseudocyst is SCA. Although classic SCA typically has thin, clear fluid; the aspirate may be bloody and thus may mimic the thin turbid to bloody aspirate of a pseudocyst. SCA fluid may contain inflammatory cells. However, pseudocyst will have a dirty proteinaceous background, will often have pigmented macrophages, and should lack any lining epithelium, as opposed to the SCA which has a bland cuboidal lining. Further differentiation can be made by analyzing cyst fluid amylase which should be high in pseudocyst and low in SCA.

MCN or IPMN with scant cyst fluid mucin may also be confused with pseudocyst in the setting of GI epithelium contamination. However, MCN and IPMN may show columnar to cuboidal cells with diffuse cytoplasmic mucin as opposed to the goblet and foveolar cells of GI contamination. Additionally, mucinous neoplastic cysts should not have a dirty proteinaceous background with pigmented inflammatory cells as PCTs do (Hamilton and Aaltonen 2010, Hruban et al. 2007, Pitman et al. 2010).

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Pancreatic Serous Cystadenoma, Cytological Findings

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Synonyms

Glycogen-rich adenoma; Serous microcystic adenoma; Serous oligocystic adenoma

Definition

Serous cystadenoma (SCA) is a nearly universally benign, cystic, epithelial neoplasm of the pancreas (Hamilton and Aaltonen 2010; Hruban et al. 2007).

Clinical Features

Hamilton and Aaltonen (2010), Hruban et al. (2007), Pitman et al. (2010), Tseng et al. (2005).

- **Incidence**
  SCA is rare and comprises 2% of all tumors of the exocrine pancreas.
- **Age**
  Older adults develop SCA with a mean occurrence in the seventh decade of life.

- **Sex**
  SCA occurs more commonly in females.
- **Site**
  SCA commonly arise in the tail of the pancreas but can arise in other locations.
- **Treatment**
  Observation is sufficient unless the cyst becomes symptomatic, becomes greater than 4 cm in size, or rapidly increases in size, in which case they are treated surgically.
- **Outcome**
  Resection is curative. Serous cystadenocarcinoma has only rarely been reported.

Macroscopy

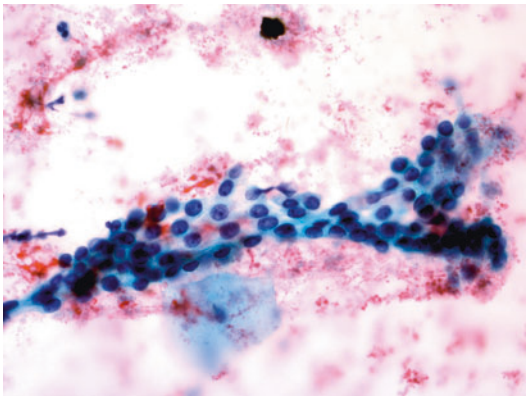
SCA is usually a well-circumscribed mass with a honeycomb-like appearance of many small cysts which is classically associated with a central, stellate scar (present in 20% of cases, especially in the microcystic variant). Because the neoplasm is composed of many small cysts, it may appear solid on imaging studies. The macrocystic and oligocystic variants contain fewer, larger cysts which creates a diagnostic pitfall with mucinous cysts of the pancreas. Unilocular and solid variants have been described (Hamilton and Aaltonen 2010; Hruban et al. 2007; Pitman et al. 2010).

Microscopy

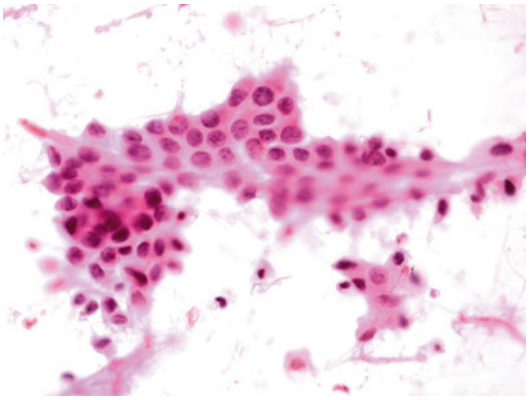
Cytology of SCA is most often non-diagnostic. The background may be clear or bloody and contain only thin, scanty mucin from gastrointestinal contamination on EUS-FNA. There may be small sheets or monolayers of bland, cuboidal cells with non-mucinous, clear, or vacuolated cytoplasm and distinct cell borders (Figs. 1 and 2) (Pitman 2010; Belsley et al. 2008; Huang et al. 2006).

Immunophenotype

Typically, SCA is positive for PAS/dPAS staining, cytokeratins, neuron-specific enolase, and



Pancreatic Serous Cystadenoma, Cytological Findings, Fig. 1 The bland cuboidal, epithelial cells of SCA with a bloody background. Papanicolaou stain, 60×



Pancreatic Serous Cystadenoma, Cytological Findings, Fig. 2 The bland cuboidal, epithelial cells of SCA with a bloody background. H&E, 60×

negative for vimentin. SCA may also be positive for Muc6, Muc1, and alpha inhibin (Hruban et al. 2007). However, FNA specimens seldom procure sufficient tissue to allow for cell block immunohistochemistry.

Molecular Features

The fluid carcinoembryonic antigen (CEA) of serous cystadenomas is generally less than 5 ng/mL, with amylase less than 250 IU/mL. KRAS expression is wildtype. There is loss of heterozygosity at 3p25 in the case of VHL

associated SCA (Pitman 2010; Belsley et al. 2008; Huang et al. 2006).

Differential Diagnosis

The differential diagnosis includes benign elements from surrounding normal structures and other cystic neoplasms with bland cytology or scant cellularity on FNA. Gastrointestinal (GI) epithelium or pancreatic ductal epithelium can have a similar bland, cytologic appearance to SCA. GI epithelium appears in large sheets of columnar cells, as opposed to the cuboidal cells of SCA. GI epithelium may also have goblet cells. Pancreatic ductal epithelium appears as honeycombed sheets and can have columnar to cuboidal cells. Both pancreatic and GI epithelium have cytoplasmic mucin, distinguishing them from SCA.

There are cystic neoplasms that can have bland cytology like SCA. Cystic pancreatic neuroendocrine tumors have salt and pepper nuclei and will stain for neuroendocrine markers. Mucinous cystic neoplasm (MCN) has columnar, mucin-containing epithelium with a mucinous background.

In the case of a scantly cellular specimen, if the fluid is filled with debris, pigmented histiocytes, and other inflammatory cells, pseudocyst is the most likely diagnosis. Conversely, thick, or mucin-rich cyst contents with columnar mucin-containing cells points to MCN or intraductal papillary mucinous neoplasm (Hruban et al. 2007; Pitman 2010; Huang et al. 2006).

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Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings

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Synonyms

Frantz tumor; Hamoudi tumor; Low grade papillary neoplasm; Papillary and solid neoplasm; Papillary-cystic neoplasm; Papillary-cystic tumor; Papillary epithelial neoplasm; Papillary-cystic carcinoma; Papillary-cystic epithelial neoplasm; Solid and cystic acinar cell tumor; Solid and cystic tumor; Solid and papillary epithelial neoplasm; Solid and papillary neoplasm; Solid-pseudopapillary tumor

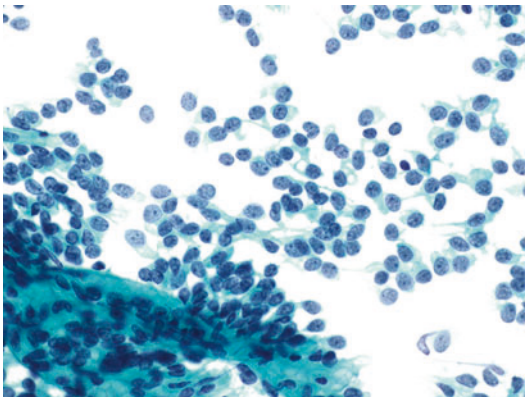
Definition

Solid-pseudopapillary neoplasms (SPNs) are rare, well-circumscribed, solid, secondarily cystic epithelial neoplasms that form pseudopapillary structures. The cell of origin is unknown.

Clinical Features

- **Incidence**
  SPNs comprise 1–2% of all pancreatic exocrine tumors (Song et al. 2012).
- **Age**
  Mean age of presentation ranges from 22 to 28 years old, but they can occur in older adults (Chakhachiro and Zaatari 2009).

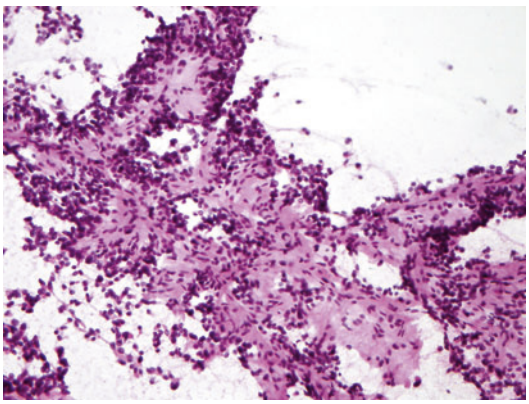
- **Sex**
  Ninety percent occur in young women (Chakhachiro and Zaatari 2009).
- **Site**
  SPNs show no site predilection within the pancreas (Chakhachiro and Zaatari 2009).
- **Treatment**
  Surgical resection is the treatment of choice, including resection of metastatic disease (Song et al. 2012; Mortenson et al. 2008).
- **Outcome**
  Ninety five percent of patients are cured with surgical resection and recurrence rates are very low. Metastases occur in 10–20% of patients, but some data suggests that even patients with metastases or unresectable disease can have good survival. There has been reported success with chemotherapy (cisplatin, 5-fluorouracil, and gemicitabine) and radiotherapy (Mortenson et al. 2008). There are rare case reports of aggressive SPNs (Song et al. 2012; Mortenson et al. 2008).



Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 1 Solid-cellular smear pattern of SPN on the right hand side with a papillary, vascular formation on the left. Papanicolaou stain, 40×

Macroscopy

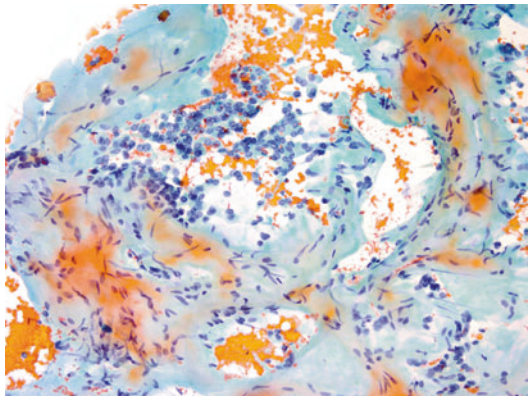
SPNs range in size from 0.5 to 34.5 cm, with a mean of 6.08 cm. They often appear solid and cystic. Larger lesions may have a fibrous pseudocapsule with a variegated, friable, and hemorrhagic cut surface. Grossly, SPNs can mimic a pseudocyst if extensive degenerative changes are present (Chakhachiro and Zaatari 2009; Hruban et al. 2007).



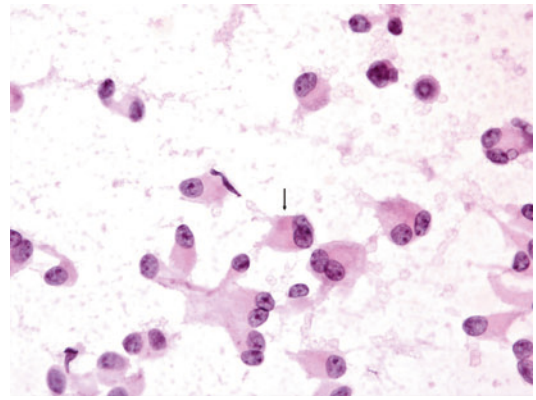
Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 2 Hyalinized vessels in a papillary pattern lined by loosely cohesive cells. H&E, 20×

Microscopy (Cytologic Features)

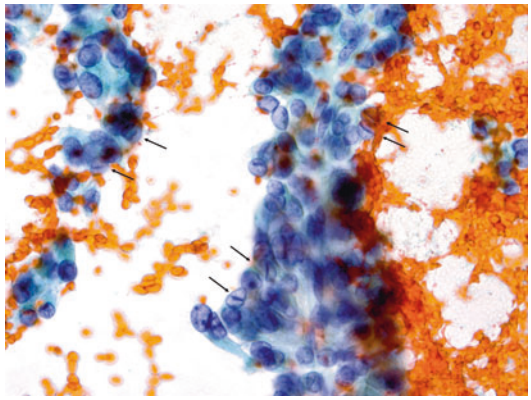
SPNs usually produce a very cellular smear with FNA, composed of discohesive, single cells (solid-cellular smear pattern) (Fig. 1). Myxoid or hyalinized vascular stalks lined by one or more layers of loosely arranged, neoplastic cells are seen (Figs. 2 and 3). Cells can sometimes form ependymal-like rosettes. Cells are bland and cuboidal with little pleomorphism and have ovoid to bean-shaped nuclei, fine chromatin, inconspicuous nucleoli, and nuclear grooves (Fig. 4). The cell cytoplasm is finely vacuolated with perinuclear vacuoles, PAS-positive, diastase-resistant hyaline globules, and indistinct cell borders (Figs. 5–8). Cystic debris (foam cells and necrotic debris) can be present in the background. Cholesterol crystals, calcifications, globules of amorphous myxoid material, and, rarely, ossification can be seen (Hruban et al. 2007).



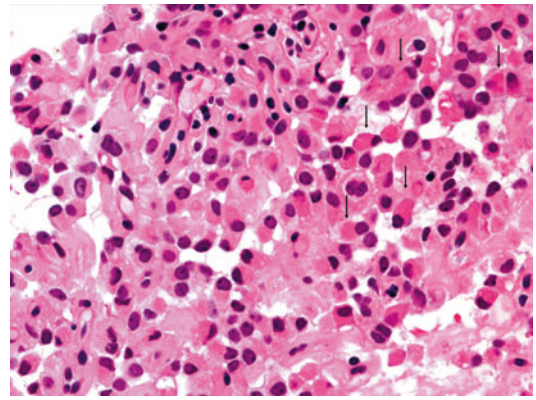
Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 3 Extremely hyalinized vessels. Papanicolaou stain, 20×



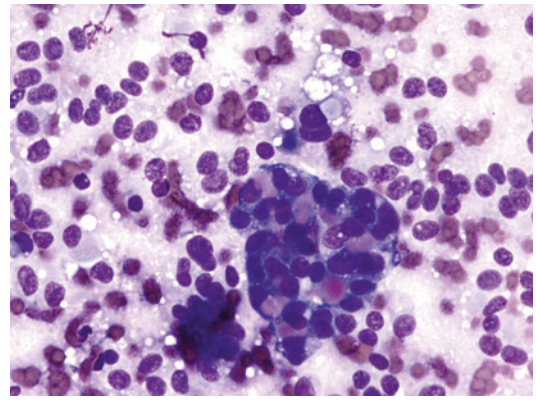
Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 5 Discohesive cell pattern. The majority of cells have poorly defined, vacuolated cytoplasms. Occasional hyaline globules are present (*arrow*) H&E, 40×



Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 4 Tumor cells are ovoid with occasional nuclear grooves (*arrows*). Papanicolaou stain, 40×



Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 6 Many hyaline globules are present in the cell block. H&E, 40×

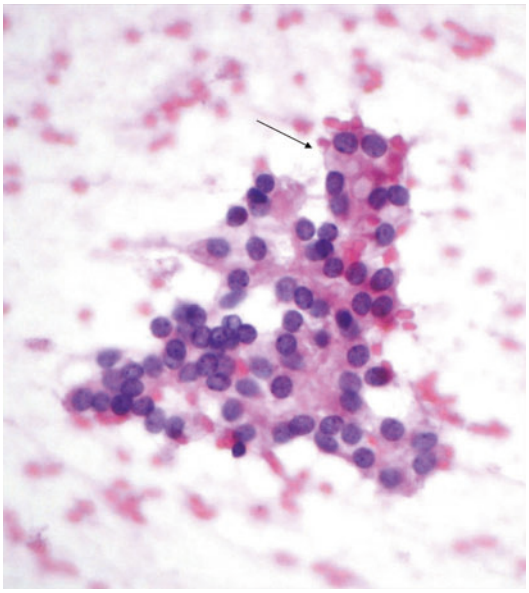


Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 7 Hyaline globules on a Diff Quik smear. 60×

Immunophenotype

Tumor cells demonstrate nuclear staining for β-catenin (which is ubiquitously positive in the cytoplasm of most cells) (Fig. 9) and is the marker of choice in confirming the diagnosis. Cells are also positive for alpha 1-antitrypsin, CD10, CD56, vimentin, α-1 antitrypsin, CD117 and progesterone receptor. Tumor cells are negative for E-cadherin membrane expression, CA19-9 and chromogranin A. Variable expression is seen with cytokeratin (AE1/AE3, CAM5.2) and other

neuroendocrine markers other than chromogranin A. Cytokeratins 7 and 19 are usually negative. PAS-D highlights the cytoplasmic hyaline globules (Chakhachiro and Zaatari 2009).



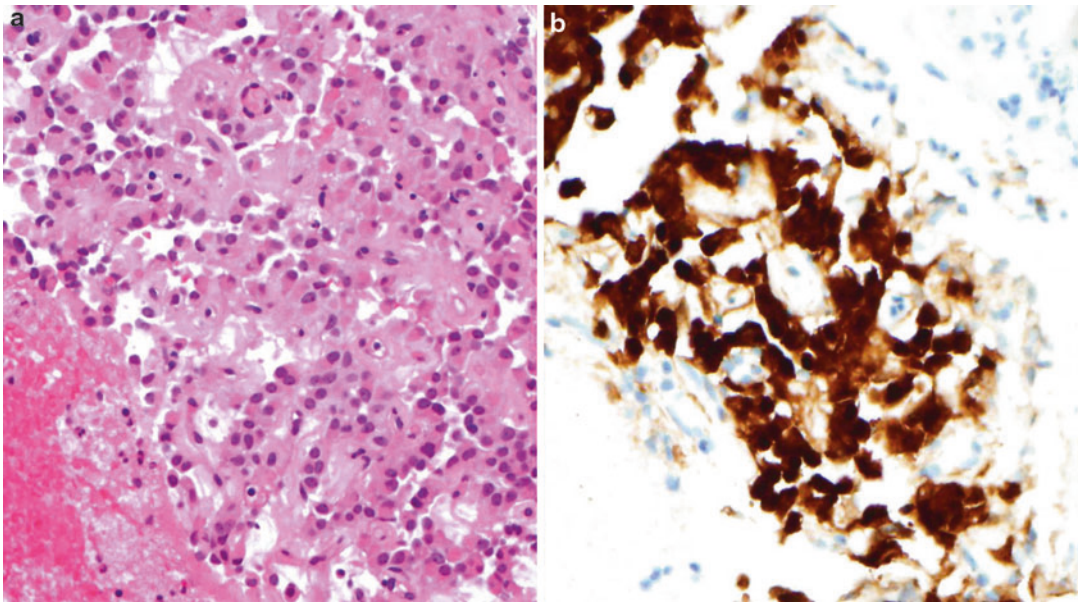
Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 8 Perinuclear vacuoles (*arrow*). H&E, 40×

Molecular Features

The majority of SPNs show a point mutation in exon 3 of the β-catenin gene. Chromosomal gains in 13q, 17q, 1q, and 8q, and losses in 11q have also been reported (Chakhachiro and Zaatari 2009).

Differential Diagnosis

The differential diagnosis is primarily with other parenchymal-rich, stromal poor neoplasms of the pancreas. Acinar cell carcinomas have more abundant, well-defined cytoplasm with zymogen granules and large nuclei with prominent nucleoli. Acinar cell carcinoma also shows acinar formation rather than papillary structures as seen in SPNs. Pancreatic neuroendocrine tumors have salt-and-pepper chromatin and also lack the papillary features of SPN. Pancreatoblastomas usually occur in young children and have acinar formations and squamous corpuscles, both features not seen in SPNs. In cases of extensive degeneration, SPNs can appear to be pseudocysts. Thorough sampling and clinical history are important for distinguishing between SPN and



Pancreatic Solid-Pseudopapillary Neoplasm, Cytological Findings, Fig. 9 SPNs are strongly positive for beta-catenin. (**a**) H&E, 40×. (**b**) Immunohistochemical stain for beta-catenin, 40×

pseudocyst in this situation (Chakhachiro and Zaatari 2009; Hruban et al. 2007).

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Papillary Carcinoma of Thyroid, Cytological Findings

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Synonyms

Papillary adenocarcinoma

Definition

Papillary carcinoma of the thyroid is a carcinoma characterized by papillary arrangements lined by usually cuboidal cells showing some typical nuclear atypia. The typical form is well known and quite easy to diagnose on histology as well as on cytology after fine needle aspiration (FNA) cytology. But recently, during the last 10 years, many things have changed in the knowledge of this cancer: (1) many variants have been described, enhancing the diagnostic difficulties; (2) due to the ultrasonographic performances, incidentalomas are more and more frequent as well as the detection of small tumors, and sometimes very small ones, less than 10 mm, called microcarcinoma, leaving us to question new surgical strategies; and (3) molecular markers have been highlighted, leading to a more specific diagnosis but also representing, for some of them, prognostic factors which have been taken into account for treatments and follow-ups. To sum up, papillary carcinoma is an old well-known cancer with quite new challenges.

Clinical Features

- **Incidence**
  Thyroid cancer represents about 4–8% of the thyroid nodules around in the world, and papillary carcinoma represents about 85% of these cancers.
- **Age**
  This cancer may occur at any time in life, even in children but with a peak at 30–40 years old.
- **Sex**
  It is more frequent in women with a female: male ratio of 3:1.
- **Site**
  Papillary carcinoma may be located anywhere in the gland; some papillary carcinomas are multifocal.
- **Treatment**
  For cases diagnosed by FNA, a total thyroidectomy is required before treatment followed by radioiodine therapy. For cases discovered incidentally after a lobectomy for a benign nodule, therapeutic decision will essentially depend on the size, TNM stage of the tumor as well as on the final histology due to the poor prognosis of some variants: In some cases, thyroid totalization and radioiodine treatment will be necessary; in other cases, for microcarcinoma, for instance, a homolateral neck lymph node dissection without totalization may be planned.

- **Outcome**
  Papillary carcinomas are well-differentiated carcinomas and their prognosis is usually good; nevertheless, papillary carcinomas/variants such as multifocal, tall cell carcinoma, columnar cell carcinoma, diffuse sclerosing or oncocytic variant have worse prognosis.

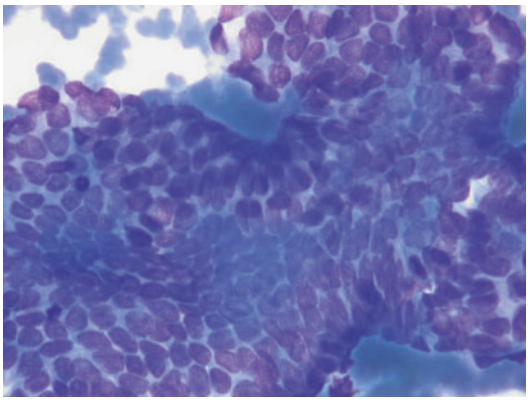
Macroscopy

Regardless of the type of resection, lobectomy, or total thyroidectomy, papillary carcinoma appears like a nodule often surrounded by a thick capsule and with a heterogeneous brilliant, cut-surface. It is gray white in color. Usually, it is a solid nodule but some cystic form may occur; those are rare. Nodules less than 10-mm wide are considered as microcarcinoma. They are very difficult to spot in the multinodular thyroid gland.

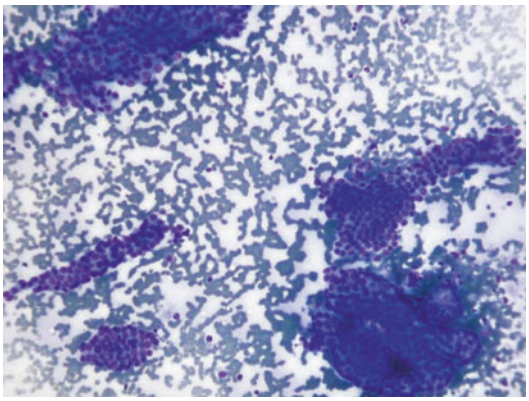
Microscopy

Classic type papillary carcinomas are represented by hypercellular aspirations. Cells are grouped either as papillae or as sheets of cells with some nuclear overlapping and a higher number of cells as usual; the sheets have straight borders (Fig. 1). Nuclei, at a low magnification, may appear quite regular, but at a higher magnification, they are enlarged, elongated, often grooved (Fig. 2). Clear pseudoinclusions may be observed, very suggestive for a papillary carcinoma but not specific (Fig. 3). Psammomas, very large giant cells with dense cytoplasm and many nuclei, are often observed; more or less lymphocytes may be seen on the background. Colloid is often absent; when present, it is presented as droplets with a metachromatic pink color on MGG staining.

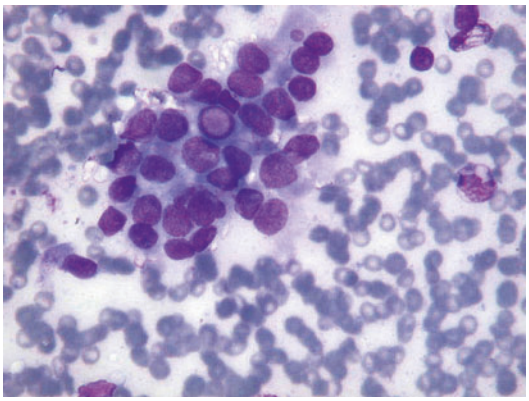
In liquid-based cytology, the same criteria may be described; nevertheless, papillae (Fig. 4) and



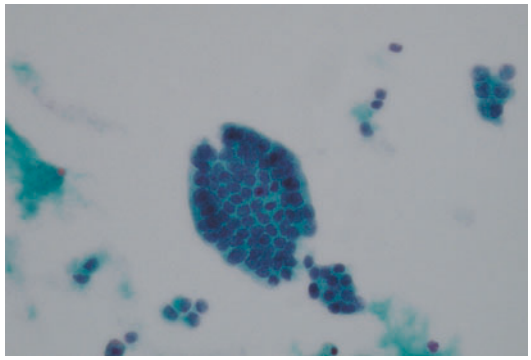
Papillary Carcinoma of Thyroid, Cytological Findings, Fig. 2 Large sheet of cells with straight borders and for the cells with enlarged and elongated nuclei. These atypia lead to a diagnosis of papillary carcinoma (conventional; MGG staining ×40)



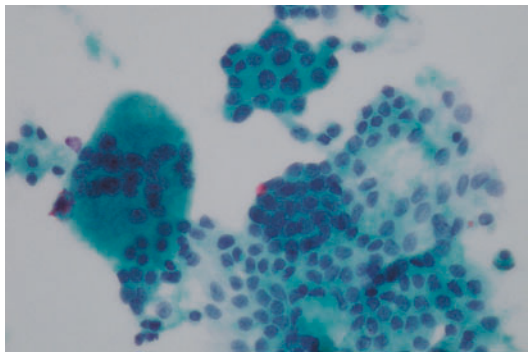
Papillary Carcinoma of Thyroid, Cytological Findings, Fig. 1 Several large sheets of cells with straight borders in a hypercellular FNA (conventional; MGG staining ×25)



Papillary Carcinoma of Thyroid, Cytological Findings, Fig. 3 One cluster of cells with enlarged nuclei and one typical pseudoinclusion (conventional; MGG staining ×40)



Papillary Carcinoma of Thyroid, Cytological Findings, Fig. 4 One papillary arrangement (LBC; Hologic®; Papanicolaou staining ×25)



Papillary Carcinoma of Thyroid, Cytological Findings, Fig. 5 One large monolayered sheet of cells with enlarged atypical nuclei and one giant histiocytic cell (LBC; Hologic®; Papanicolaou staining ×40)

pseudoinclusions are rarely observed; on the counterpart, the nuclear atypia are easy to recognize due to the optimal cells preservation and essential for the diagnosis. With the Papanicolaou staining, there is no metachromatic colloid (Fig. 5).

The follicular variant of the papillary carcinoma is the most frequently observed variant; its diagnosis is challenging due to the lack of papillae and usually of pseudoinclusions; the nuclear atypia are less marked; therefore, due to the presence of microfollicles only, this variant is often classified on FNA as a "follicular lesion of undetermined significance" or as a "follicular neoplasm" in the Bethesda System 2009; the tall cells and columnar cells variants are rare; the typical nuclear atypia have to be found in order to assert the diagnosis; the tall cells present a large cytoplasm which is often basophilic and cylindrical or spindle-shaped; pseudoinclusions are usually observed; and the columnar cells often present clusters of cells with stratified nuclei and few typical papillary carcinoma atypia. The oncocytic variant may be diagnosed if nuclear atypia are found.

Immunophenotype

The follicular cells are strongly positive for thyroglobulin and TTF1 and for many other antibodies such as CK19, Galectin-3, HBME1, CD44v6, cyclin D1, CD15, and RET oncogene. The positivity of more than a single marker is required since some benign lesion may also be positive for one antibody: For example, CK19 is often clearly positive in Hashimoto thyroiditis' follicular cells.

Molecular Features

RET/PTC oncogene and BRAF V600 mutations are quite specific for papillary carcinoma; RET oncogene is highly specific but insufficiently sensitive; BRAF V600 mutation is found in about 60% of the papillary carcinoma; furthermore, some recent articles in the literature have also underlined that the mutated papillary carcinomas are more likely to have a bad prognostic than the ones that are without mutations.

Differential Diagnosis

For the typical papillary carcinoma, the only risk of error may be due to a nondiagnostic FNA, sometimes for some nodule characteristics, for instance, if the nodule is quite entirely calcified, or in the rare cases of cystic papillary carcinoma.

For variants, the risk is essentially to classify the lesion in another category of the Bethesda System as explained above.

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Papillary Renal Cell Carcinoma, Cytological Findings

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Synonyms

Chromophilic renal cell carcinoma; Papillary renal cell carcinoma

Definition

Papillary renal cell carcinoma is a carcinoma with at least 50% of a papillary or tubulopapillary architecture. There are two recognized types: type I and type II.

Clinical Features

- **Incidence**
  Most cases are sporadic, but a hereditary papillary renal cell carcinoma (HPRCC) has been recently recognized, with an autosomal dominant transmission and conferring the patient to a higher risk of multiple microscopic bilateral renal tumors. Sporadic papillary renal cell carcinoma (PRCC) represents 10% of all renal cell tumors. Hereditary forms are very rare, and the number of people and families who have HPRCC is unknown.
- **Age**
  Hereditary forms affect patients in the range of 35–55 years, and sporadic cases have an age distribution that is similar to clear cell carcinoma of 52–66 years.
- **Sex**
  Men are mainly affected with an M/F ratio 1.8:1 to 3.8:1.
- **Site**
  Seventy percent of the tumors are limited to the kidney. Type I PRCC is usually diagnosed in a lower stage than type II.
- **Outcome**
  Survival depends whether we are considering a type I or type II tumor. Type II is more aggressive with higher nuclear grade and is generally diagnosed in more advanced stages than type I and is associated with worse prognosis. Important prognostic factors beyond histology are stage at presentation and the presence of sarcomatoid differentiation. Global 5-year survival ranges from 49% to 84% (Delahunt and Eble 2004).

Macroscopy

At section the tumor has a brown tan color with large areas of hemorrhage and necrosis. Comparing to other renal cell carcinoma (RCC), this PRCC is more frequently bilateral and multifocal.

Microscopy

Two histological types are recognized: type I and type II.

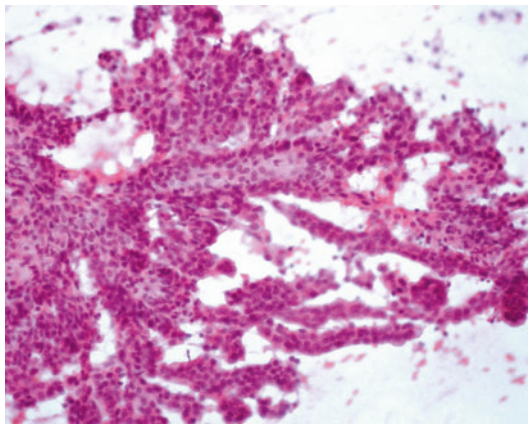
Hereditary forms are more prone to have type I PRCC.

Type I PRCC is composed by short and delicate papillae covered by one or two layers of small cubic epithelial cells (Fig. 1). Neoplastic cells have scant clear cytoplasm and low-grade (Fuhrman) nuclei. Characteristically, neoplastic cells as well as macrophages display intracytoplasmic hemosiderin granules (Figs. 2 and 3). Papillae are edematous, sometimes with a cystic-like appearance, or they can present psammoma bodies inside their core. In the background necrosis and hemorrhage are frequent as are macrophages disposed in aggregates or within the cores of papillae (Fig. 2) (Ugalde and López 2008).

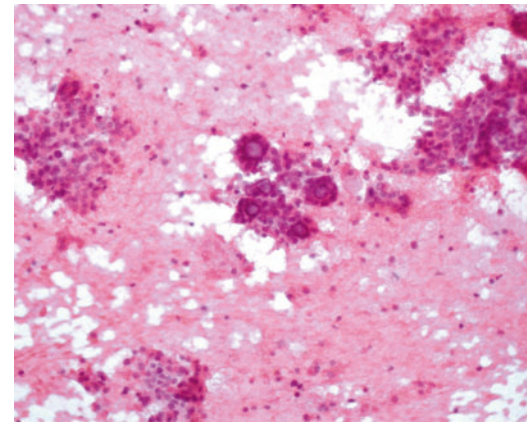
Type II PRCC is composed of papillae with dense and large fibrous cores, covered by large stratified eosinophilic cells with generally higher nuclear grade.

There have been described solid variants where short papillae and tubules are densely packed. Sarcomatoid differentiation can exist in approximately 5% of the cases.

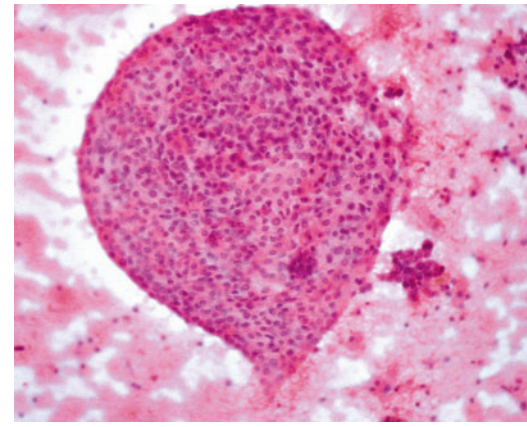
In cytology foamy macrophages and intracytoplasmic hemosiderin are a guide mark for the diagnosis of PRCC. Malignant cells are arranged in papillary groups covering fibrovascular cores. Psammoma bodies are common. In type I, nuclei are small, bland sometimes with grooves (Geisinger et al. 2003; Puttaswamy et al. 2006). Nucleoli and pleomorphism are prominent type II tumors. In type II, neoplastic cells display abundant and eosinophilic cytoplasm (Geisinger et al. 2003; Puttaswamy et al. 2006).



Papillary Renal Cell Carcinoma, Cytological Findings, Fig. 1 Remark a nice papillary architecture (H&E 200×)



Papillary Renal Cell Carcinoma, Cytological Findings, Fig. 2 There is a hemorrhagic background with dispersed macrophages and few papillary groups



Papillary Renal Cell Carcinoma, Cytological Findings, Fig. 3 Hemosiderin-laden cytoplasm in neoplastic cells (H&E, 200×)

Immunophenotype

Papillary renal cell carcinomas are positive for racemase, cluster differentiation CD10, and keratin 7. Type II is less frequently positive for cytokeratin 7. Type I is also positive for Mucin 1, cell surface associated (MUC1) and type II for E-cadherin.

Molecular Features

- Papillary renal cell carcinoma is characterized by loss of Y chromosome in men, trisomy or tetrasomy of chromosomes 7 and 17, and in a lesser percentage with trisomy of chromosomes 16, 20, and 12, by order of frequency.

Differential Diagnosis

Metastasis from other papillary carcinomas to the kidney must be ruled out by immunocytochemistry. Differential diagnosis should be done with clear cell renal carcinoma with papillary features. The presence of foamy macrophages and intracytoplasmic hemosiderin is according to some authors the two most sensitive criteria for papillary renal cell carcinoma diagnosis. Clear cell renal cell carcinoma is negative in 80% of the cases for cytokeratin 7.

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Papillary Tumors of the Breast, Cytological Findings

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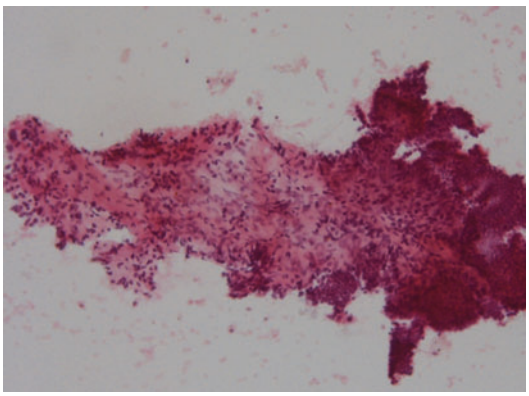
Synonyms

Papillary ductal breast lesions

Definition

Whether benign or malignant, a papillary lesion is defined by papillary structures and branches supported by a fibrovascular scant or prominent stalk. A papillary lesion may be benign or malignant. The benign group includes solitary papilloma, papillomatosis, and sclerosing papilloma. Malignant diagnoses include mainly papillary carcinoma (Fig. 1).



Papillary Tumors of the Breast, Cytological Findings, Fig. 1 Papillary lesion. Observe fibrous and thick stromal core. A papilloma was diagnosed in the surgical excision (H&E stain)

Clinical Features

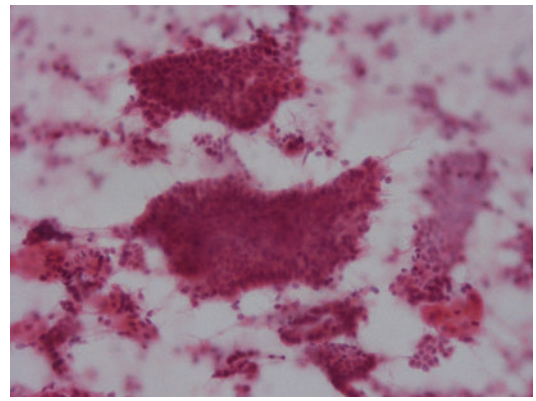
- **Incidence/Age/Sex**
  Papillary lesions occurred in patients with a wide age range, including children and adolescent females. Papillomas represent 10% of all benign lesions, whereas papillary carcinomas represent 0.3% of all breast carcinoma and are rare prior to the age of 30 years old. This carcinoma often occurs in postmenopausal women. Papillary carcinoma represents 1% of all breast carcinomas in men.
- **Site**
  Papilloma is usually centrally located solitary lesion associated with nipple discharging. The lesions are usually less than 2.5 cm in diameter. Papillary carcinoma either noninvasive or invasive is also centrally located.
- **Treatment/Outcome**
  Because different papillary lesions may have variable morphological appearance, a complete excision is mandatory if a papillary lesion is detected. Solitary papillary carcinoma should be excised with a rim of normal breast parenchyma whereas multifocal cancers should be approached as carcinoma in situ. Papillomatosis and papillomas require a cautious approach and consideration of family history and mammographic findings.

Macroscopy

Benign papilloma and papillary carcinoma cannot be distinguished on the basis of gross appearance. In the majority of cases, duct papillomas vary from few millimeters to >5 cm. The majority of papillary carcinomas are well demarcated usually soft and around 1–3 cm in its diameter (Fig. 2).

Microscopy

Aspiration cytology of papillary lesions might be interpreted with caution. The typical feature includes three-dimensional papillary groups with high-columnar cells and hemosiderin-laden macrophages. The NCI-sponsored conference for revising guidelines for breast FNA placed these papillary lesions into an indeterminate category due to the non-univocal criteria for a correct classification. Cytological features that favor papillary carcinoma are represented by high monomorphic cellularity organized in complex branching, with discohesive columnar cells with atypia and hyperchromasia. Also the fibrous stromal cores are different between benign and malignant papillary lesions: thicker and small fibrous in papilloma whereas complete and thin in carcinomas. The features favoring papilloma might be summarized in (1) tight three-dimensional papillary cluster of typical cells with occasionally spindle-shaped stromal cells, (2) evidence of columnar cells, (3) stromal cores, and (4) foam cells (Fig. 3).



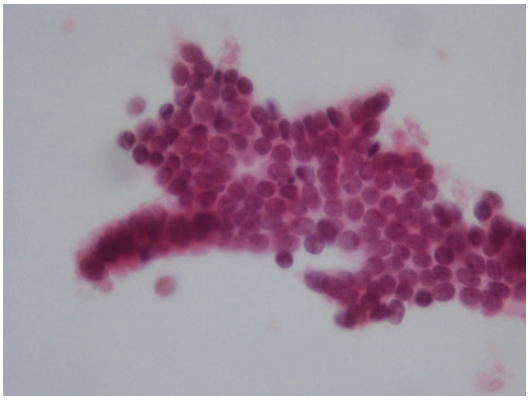
Papillary Tumors of the Breast, Cytological Findings, Fig. 2 Papillary lesion. Presence of three-dimensional papillary cluster of cells. Note apocrine cells (H&E stain)

The features favoring papillary carcinoma might be summarized in (1) three-dimensional papillary cluster of atypical cells, (2) evidence of tall columnar cell component, (3) naked and isolated atypical nuclei, and (4) blood diathesis and hemosiderin macrophages.

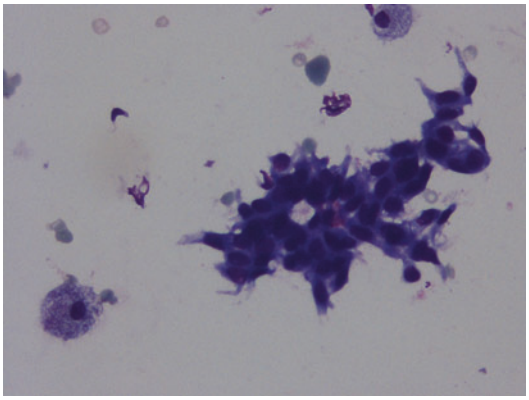
Both FNA and core biopsy share the same diagnostic power to separate benign form malignant papillary lesions.

Immunophenotype

Immunostains for myoepithelial component are helpful in characterizing these papillary entities.



Papillary Tumors of the Breast, Cytological Findings, Fig. 3 Papillary lesion. Cluster of cells with presence of columnar cells (H&E stain)



Papillary Tumors of the Breast, Cytological Findings, Fig. 4 Columnar cells and macrophages in FNA of a papillary lesion of the breast (Giemsa stain)

Papillary carcinomas share the absence of myoepithelial component expressed by negativity for p63, calponin, or CD10 and expression of keratins 5/6 and 14. It has been suggested that CD44 might be able to distinguish benign papillomas from carcinomas. The expression was evident in 70% of normal cells and papillomas whereas papillary carcinomas express in only 10% of cells. According to our experience, in cytological smears p63 is the best marker because it highlighted the nuclei even if the cytoplasm was lost during the smearing (Fig. 4).

Molecular Features

Clonal analysis of solitary papilloma has shown that it is monoclonal in its origin, suggesting a derivation from a common precursor cell capable of differentiating into epithelial and myoepithelial components. Higher frequency of activating point mutations of PIK3CA, AKT1, and RAS family genes was found in benign papilloma than in papillary carcinoma. Loss of heterozygosis on chromosome 16p13 in the TSC2/PKD1 gene region was found in benign papillary lesions as well as in malignant. The loss of heterozygosis on chromosome 16q23 with the D16S476 was found only in malignant papillary lesions.

Differential Diagnosis

Although is not so difficult to recognize a papillary lesion in a breast FNA, in general it is not possible to conclude if the lesion is benign or malignant. Sometimes, epithelial proliferative lesions and fibroadenomas can show columnar cells in the smears.

References and Further Reading

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Penile Neoplasias, Cytological Findings

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Definition

Penile neoplasias include Penile intraepithelial neoplasia (PeIN) as premalignant lesions and malignant neoplasms (squamous cell carcinoma, melanoma, basal cell carcinoma, adenocarcinoma – Paget disease of the penis, sarcoma. . ..).

Epidemiology

The incidence of penile neoplasias is related to the prevalence of HPV in the population. Penile cancer is rare in Europe, present in less than one man in 100,000, and accounts for less than 1% of cancers in men in the USA. Penile cancer is, however, much more common in some parts of Asia, Africa, and South America (American Cancer Society 2016).

Penile Intraepithelial Neoplasia (PeIN)

***Definition and introduction*:** Penile intraepithelial neoplasia (PeIN) is a premalignant lesion that can affect any part of the penile surface. PeIN shows different degrees of dysplasia and is therefore classified into PeIN 1, 2, and 3. All grades of epithelial dysplasia or PeIN are histological prestages of *carcinoma* in situ that may gradually lead to penile squamous cell carcinoma (SCS). PeIN 3 is also known as carcinoma in situ. PeIN is believed to have a close association with human papilloma virus infection. Treatment of PeIN can potentially prevent progression to penile cancer (Zreik et al. 2013).

Premalignant penile lesions can be broadly divided into those related to HPV infection and those that are not HPV related but caused by chronic inflammation. HPV-related lesions include Bowen's disease (BD), erythroplasia of Queyrat (EQ), and Bowenoid papulosis (BP), which are associated with "high-risk" HPV types 16 and 18. Low-risk HPV types 6 and 11 are associated with other premalignant lesions, such as giant condylomata acuminate (GCA) or Buschke-Lowenstein tumours. Non-HPV-related lesions are primarily linked to genital lichen sclerosus et atrophicus (LS). However, they are also associated with rarer chronic inflammatory conditions such as penile cutaneous horn, leukoplakia, and pseudoepitheliomatous, keratotic micaceous balanitis (PKMB) (Algaba et al. 2002; Backes et al. 2009; Barbagli et al. 2006).

A recent reclassification system based on cell morphology, squamous differentiation, and pathogenesis has been suggested. In the new proposed classification system the term penile intraepithelial neoplasia (PeIN) is used to describe all premalignant lesions. It is further subclassified into differentiated PeIN, the subtype most frequently associated with chronic inflammation and not HPV, and three other subtypes (warty, basaloid, and mixed warty-basaloid), which are linked to HPV infection (Chaux et al. 2010; Wikström et al. 2012; Cubilla et al. 2000).

***Pathology*:** Morphological criteria for differentiated PeIN included the presence of acanthosis, abnormal epithelial maturation with cellular atypia at the basal/parabasal regions or above in the epithelium, enlarged keratinocytes with abundant eosinophilic cytoplasm, prominent intercellular bridges, vesicular nuclei, and occasional prominent nucleoli, hyperkeratosis/hypergranulosis, elongation of rete ridges, and intraepithelial keratin pearl formation. Among the undifferentiated variants, in basaloid PeIN, the epithelium was replaced by a monotonous population of small- to intermediate-sized, round

to ovoid, rarely spindle, immature cells with high nuclear/cytoplasmic ratio, abundant mitosis and apoptotic bodies, flat or slightly irregular epithelial surface, and abrupt parakeratosis; focal koilocytosis was acceptable.

Warty PeIN was characterized by a thickened epithelium with undulating/spiking papillary surface, parakeratosis, cellular pleomorphism and anaplasia, conspicuous, often pleomorphic, koilocytosis, and dyskeratosis. Warty-basaloid PeIN showed overlapping features of both warty and basaloid types, with basaloid-type cells in the bottom half and warty features at the surface (Cubilla et al. 2000).

***Cytology findings*:** Mild dysplasia (PeIN grade I). Cytological atypia is generally slight with only mild pleomorphism of cells or nuclei. Cytological samples are brush and scarf. Corresponding cytologic samples show abnormal cells with mature or superficial-type cytoplasm. Low-grade lesion typically involves squamous cells with mature, intermediate, or superficial-type cytoplasm with well-defined polygonal cell borders. Nuclear enlargement is at least three times the size of a normal intermediate cell nucleus (Wikström et al. 2012). Although the nucleus is hyperchromatic, the chromatin is distributed uniformly or it may appear degenerated and smudged if associated with cytopathic changes induced by HPV. Cells with HPV cytopathic effect (koilocytes) are also interpreted as low-grade dysplasia or PeIN I on cytologic smears. In these cases, the nucleus may not be enlarged, but is usually hyperchromatic and "wrinkled" (Wikström et al. 2012).

Moderate dysplasia (PeIN grade II) demonstrates a proliferation of atypical cells extending into the middle third of the epithelium. The cytological changes are more severe than in mild dysplasia and changes such as hyperchromatism and prominent cell and nuclear pleomorphism may be seen. Increased and abnormal mitoses may be present, but these are usually located in the basal layers. Cytologically moderate dysplasia shows cells with less cytoplasm, larger nuclei, and occasionally with asymmetrical nuclear outlines. The chromatin is increased and granular (Wikström et al. 2012) (Fig. 1).

In severe dysplasia (PeIN grade III) there is abnormal proliferation from the basal layer into the upper third of the epithelium. These changes are seen in cytologic smears as immature cells, with scant cytoplasm and a high nuclear-cytoplasmic ratio. The abnormal cells can be seen singly or in crowded, dark sheets/groups (Wikström et al. 2012) (Fig. 2).

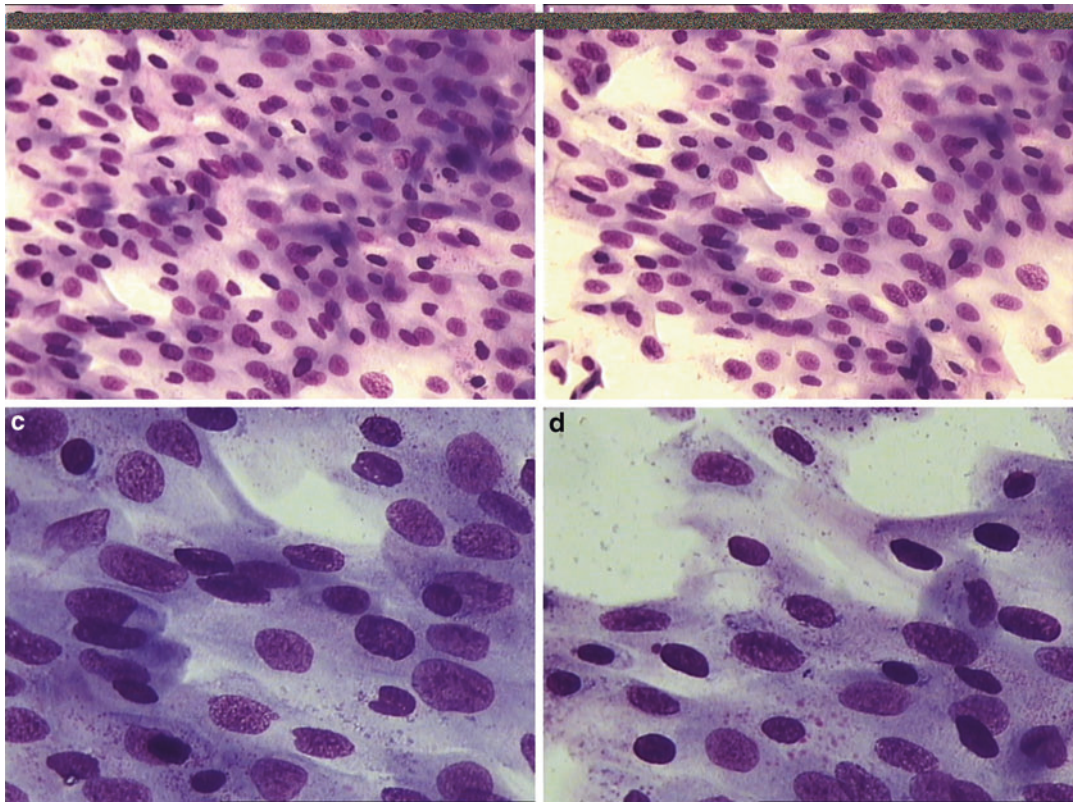
***Differential diagnosis*:** Balanitis, psoriasis, contact dermatitis, and eczem are chronical skin conditions characterized by skin inflammation and irritation without cellular atypia, lichen planus, lichen sclerosus and Erythroplasia of Queyrat show hyperkeratosis, chronic inflammations (infiltrate lymphocytes, plasma cells), and mild to moderate dysplasia with or without cytopathic changes induced by HPV, and Zoon's balanitis looks similar to PeIN grade III but have dense plasma cell infiltrate.

The differential diagnosis between penile intraepithelial lesions can be difficult. At low power view, warty lesions are papillary and basaloid lesions are flat. Cytologically, the former is more keratinized and pleomorphic, with conspicuous koilocytosis, whereas in basaloid PeIN, there is a uniform small, nonkeratinizing cell pattern. Basaloid PeIN should also be distinguished from flat urothelial carcinoma in situ of distal urethra, which may secondarily involve the penile meatal region. Squamous cell carcinoma is the most severe form of epithelial dysplasia.

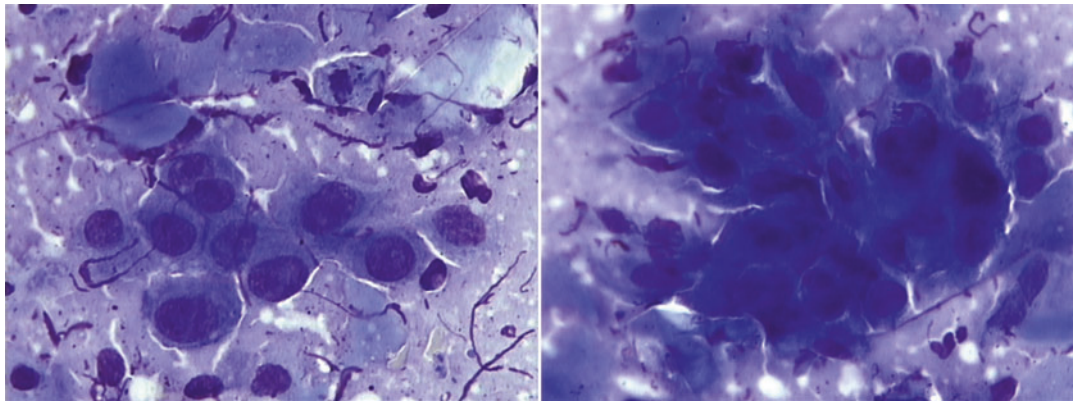
Squamous Cell Carcinoma (SCC)

***Definition*:** SCC is the most frequent penile cancer, usually originates from the epithelium of the glans or the foreskin (Hakenberg et al. 2014), and can develop anywhere on the penis. Around 95% of penile cancers are SCCs. Early stage of SCC is carcinoma in situ (CIS), without invasion by neoplastic squamous cells into the deeper tissues (stroma) of the penis.

***Pathology*:** Squamous cell carcinoma originates in epidermis, squamous mucosa, or areas of squamous metaplasia. In skin, it arises from the epithelial cells of the epidermis as a small grey or brownish lesion with irregular margins to exophytic tumour or ulcer. The diagnosis of cSCC is primarily based on clinical features. A biopsy or excision and histologic confirmation should be performed in all clinically suspicious lesions in



Penile Neoplasias, Cytological Findings, Fig. 1 Penile intraepithelial neoplasia (PeIN grade II). Cytological atypia is generally slight with only mild pleomorphism of cells or nuclei (Dermal swab of leukoplakia on the penis, May-Grünwald Giemsa, (**a**, **b**) ×400 and (**c**, **d**) ×1000)

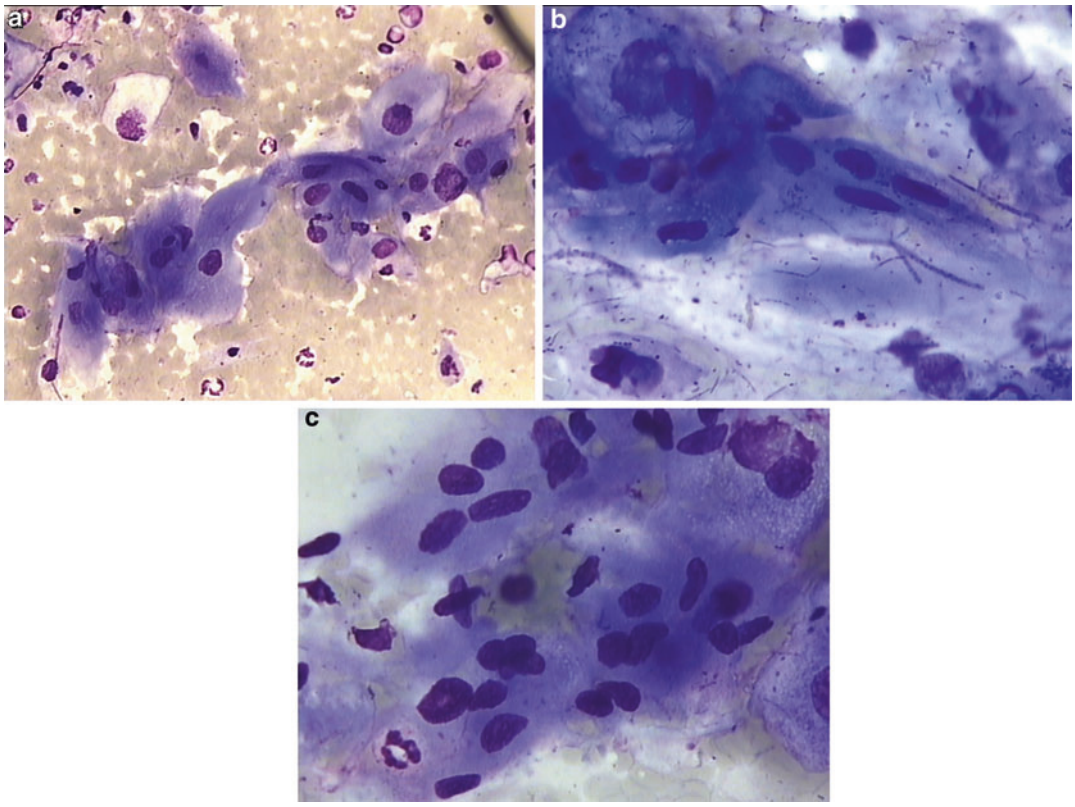


Penile Neoplasias, Cytological Findings, Fig. 2 Penile intraepithelial neoplasia (PeIN grade III). In severe dysplasia with changes are seen in cytologic smears as immature cells, with scant cytoplasm and a high nuclear-cytoplasmic ratio (Dermal swab of the penis, May-Grünwald Giemsa ×1000)

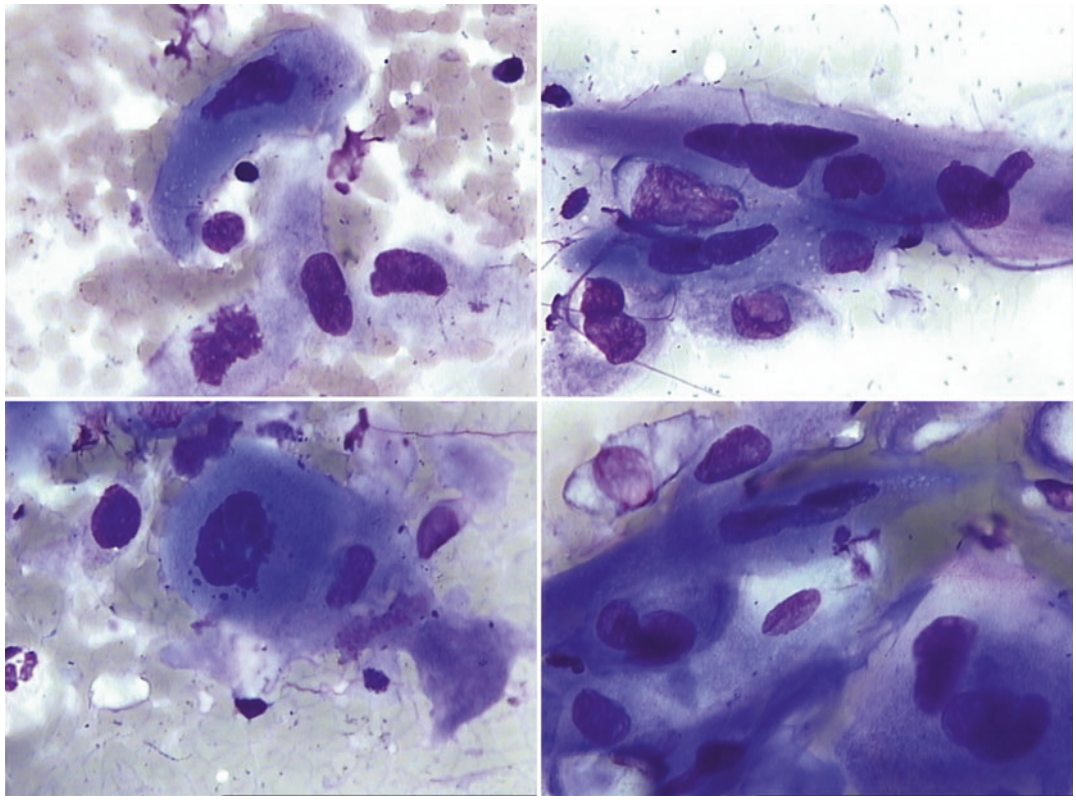
order to facilitate the prognostic classification and correct management (Stratigos et al. 2015). The histologic subtype has also been considered as a factor in determining the prognosis. Several

histologic subtypes of SCC are described, including keratoacanthoma, acantholytic, spindle cell, verrucous, clear cell, papillary, signet ring, pigmented, and desmoplastic SCC. These variants of SCC are reviewed for their clinical and histologic features and the risk of recurrence and metastasis (Rinker et al. 2001).

***Cytology findings*:** Bowen disease (BD), squamous cell carcinoma in situ (SCCIS), is the most severe form of epithelial dysplasia. When the cytological changes are very marked this may indicate that a lesion should be upgraded. Features that favor a high-grade lesion include increased numbers of abnormal cells, higher nuclear/cytoplasmic ratios, greater irregularities in the outline of the nuclear envelope, coarsening of nuclear chromatin, and chromatin clumping. Cell size, overall, is smaller in carcinoma in situ. They have a more immature type of cytoplasm that can be lacy and delicate or dense/metaplastic with rounded cell borders. At times it may not be possible to exclude the possibility of invasive carcinoma in such cases (Wikström et al. 2012). SCC of the penis, similarly to the cervix, vagina, vulva, or oropharynx, varies from well-differentiated keratinizing tumors to anaplastic carcinomas with scant keratinization. Most tumors are highly keratinized and moderately differentiated with bizarre enlarged cells including tadpole and fiber cell forms with hyperchromatic and irregular nuclei in a hemorrhagic and necrotic background (Figs. 3, 4, 5, and 6). Poorly differentiated carcinomas have variable amounts of spindle or giant cell with nonkeratinized cytoplasm



Penile Neoplasias, Cytological Findings, Fig. 3 Well-differentiated squamous cell carcinoma. (**a**–**c**) Elongated cells including tadpole and fiber cell forms in a hemorrhagic background (Swab of the shaft penis, May-Grünwald Giemsa ×400)



Penile Neoplasias, Cytological Findings, Fig. 4 Well-differentiated squamous cell carcinoma. Large hyperchromatic elongated or poligonal atypical cells with hyperchromatic irregular nuclei and abundant cytoplasm (Swab of the shaft penis, May-Grünwald Giemsa ×1000)

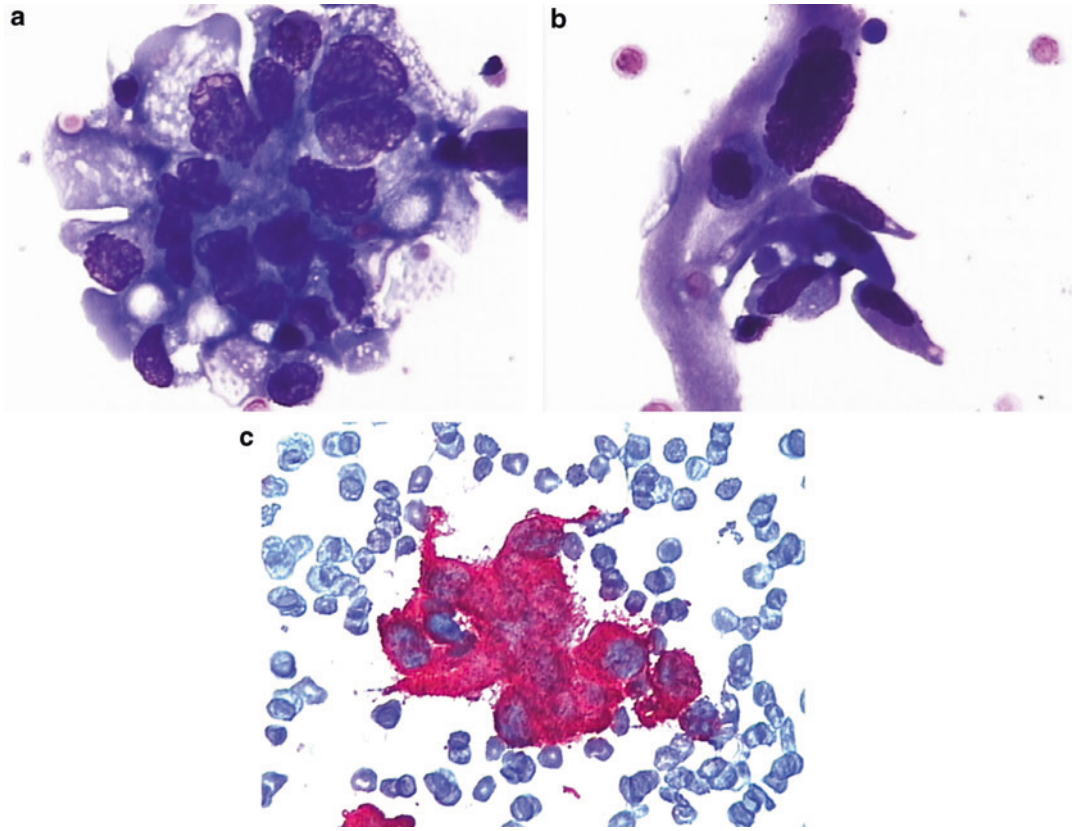
and prominent nucleoli (American Cancer Society 2016; Kocjan et al. 2013) (Fig. 7).

***Differential diagnosis*:** Squamous cell carcinoma is an invasive malignant neoplasm. Depending on the degree of cell differentiaton, cells are bizarre large, elongated, and hyperchromatic or multinucleated admixed in hemorrhagic, necrotic background (Gray and Kocjan 2010). Bowen disease is a squamous cell carcinoma in situ (SSCIS) showing epidermal dysplasia with an intact basement membrane (Barad et al. 2014). Atypical keratinocytes have less cytoplasmic and/or nuclear pleomorhism without hemorrhagic and necrotic background.

Basal Cell Carcinoma (BCC)

***Definition*:** BCC is the cancer that develops from basal cells in the deepest layer of the skin. It is a very slow growing cancer and very rarely spreads to distant parts of the body. Basal cell carcinoma of the penis is an extremely rare entity, accounts 0.03% of all BCCs in men, with very good prognosis (Roewe et al. 2014).

***Pathology*:** Basal cell carcinoma (BCC) is the tumor that affects mainly photoexposed areas, most often in the head, and seldom appears on genitalia and perigenital region (Dourmishev et al. 2013). The tumour infiltrates tissues in a three-dimensional fashion through the irregular growth of subclinical finger-like outgrowths which remain contiguous with the main tumour mass. Clinical appearances and morphology are diverse and include nodular, cystic, superficial, morphoeic (sclerosing), keratotic, and pigmented variants. Common histological subtypes include nodular (nBCC), superficial (sBCC), and pigmented forms in addition to morphoeic, micronodular, infiltrative, and basosquamous variants



Penile Neoplasias, Cytological Findings, Fig. 5 Squamous cell carcinoma. (**a**, **b**) Metastatic squamous cell carcinoma of the penis to iliac crest (Bone marrow aspirate of iliac crest, May-Grünwald Giemsa ×1000); (**c**) Immunocytochemistry. Pan-cytokeratin positive cells (LSAB ×100)

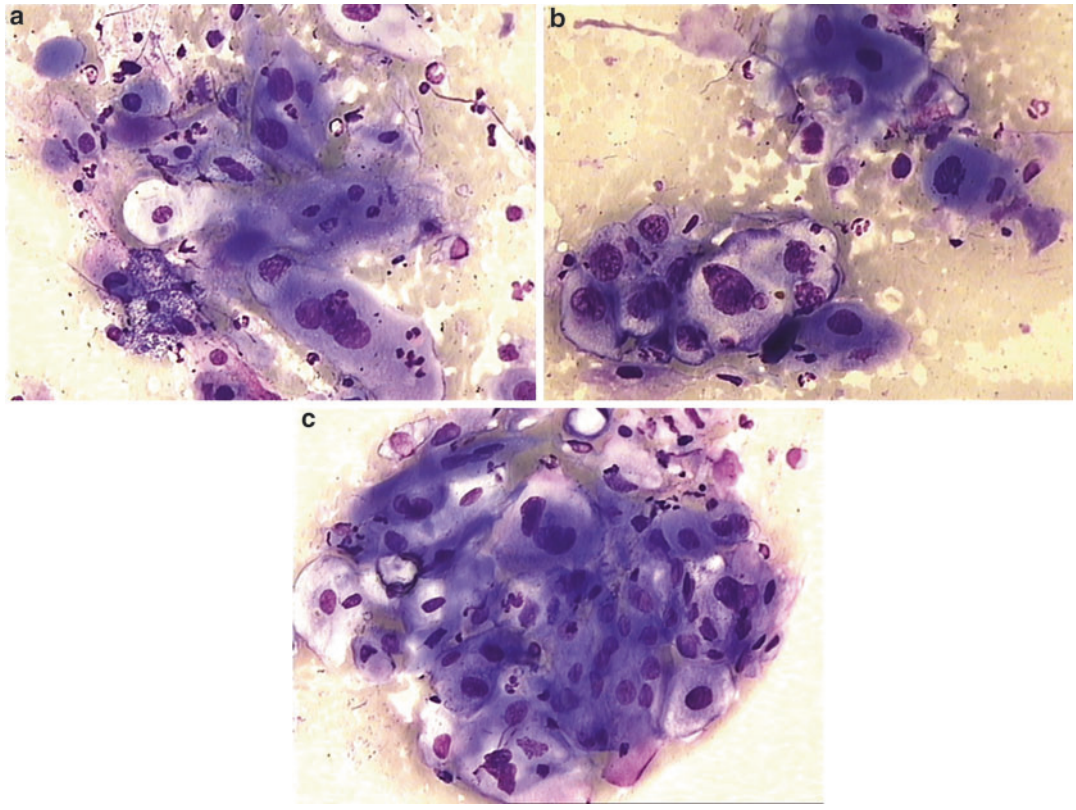
that are particularly associated with aggressive tissue invasion and destruction. Perivascular or perineural invasion are features associated with the most aggressive tumours (Telfer et al. 2008). The currently most favoured classification is one based predominantly on histological growth pattern. This classification contributes to the useful concept of low- and high-risk histological subtypes of BCC (Saldanha et al. 2003).

***Cytology findings*:** Smears of BCC are very cellular with cohesive sheets of small uniform cells without variation in nuclear size and shape (Fig. 8). Nuclei are hyperchromatic round or oval and have granular chromatin. Cytoplasm is scanty with indistinct cell borders (Kocjan et al. 2013) (Figs. 9 and 10).

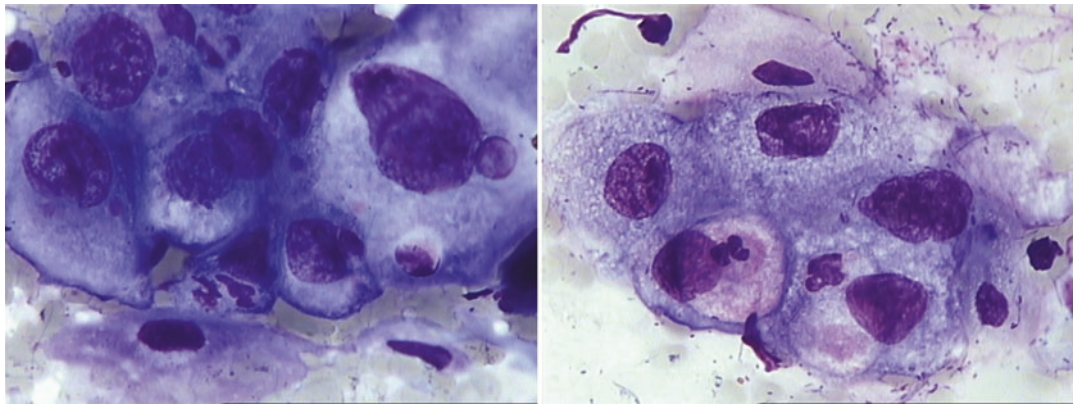
***Differential diagnosis*:** Tumors with basaloid cells may be mistaken for the basal cell carcinoma as a pilomatrixoma or sebaceous gland tumors. In case of pilomatrixoma, it helps clinical presentation, "ghost" cells, and multinucleate giant cells. Merkel cell carcinoma has greater cellular dissociation. Metastatic small cell carcinoma has shown moulding efect and sometimes necrotic background (Gray and Kocjan 2010).

Adenocarcinoma: Paget Disease

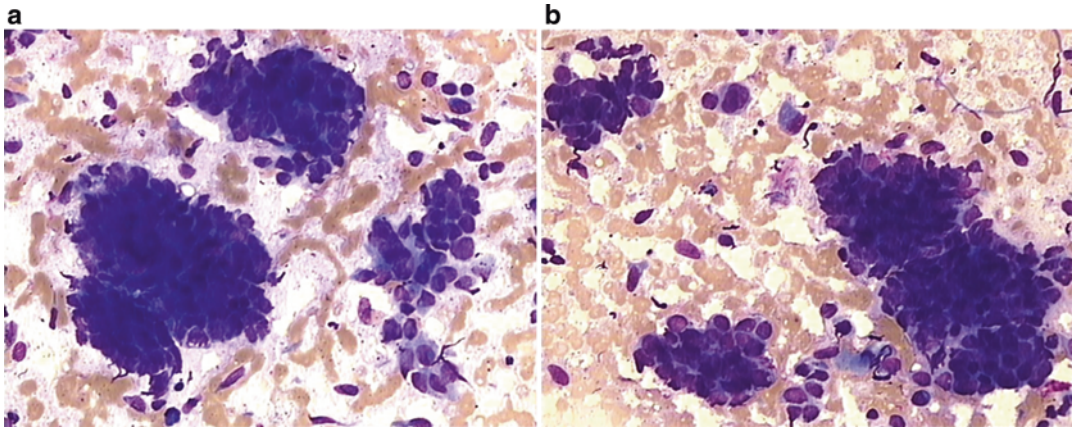
***Definition*:** Adenocarcinoma – Paget disease is the cancer of the glandular cells that produce sweat in the skin of the penis, similarly to the perineum, vulva, axilla, and scrotum. It is a rare cutaneous, intraepithelial adenocarcinoma of the epidermis, which sometimes infiltrate into the underlying dermis. Isolated Paget's disease of the penis is extremely rare.



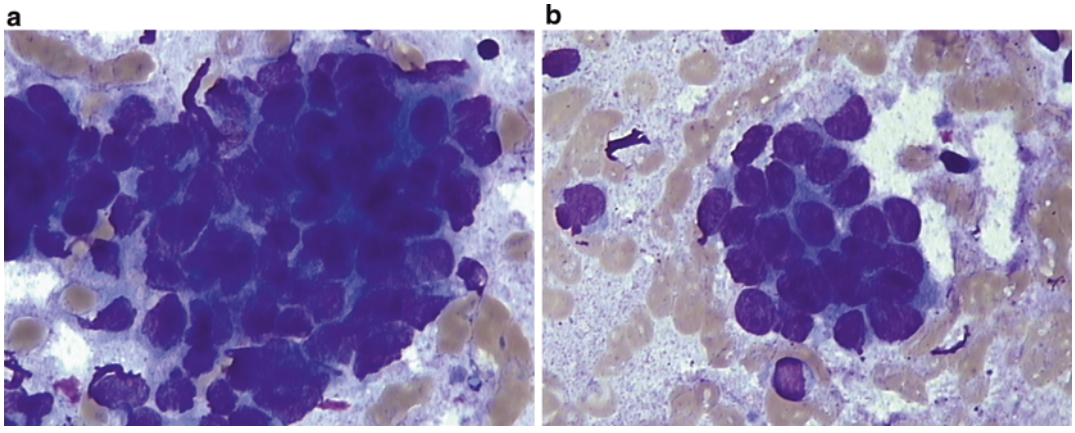
Penile Neoplasias, Cytological Findings, Fig. 6 Squamous cell carcinoma. (**a**–**c**) Bizarre enlarged cells including tadpole and fiber cell forms with hyperchromatic and irregular nuclei in a hemorrhagic and necrotic background (Swab of the glans penis, May-Grünwald Giemsa ×400)



Penile Neoplasias, Cytological Findings, Fig. 7 Poorly diferentiated squamous cell carcinoma. Large poorly diferentiated atypical cells with irregular nuclei (Swab of the glans penis, May-Grünwald Giemsa ×1000)



Penile Neoplasias, Cytological Findings, Fig. 8 Basal cell carcinoma. (**a**, **b**) Clusters of small uniform cells and many background stripped nuclei (Dermal swab of the penis, May-Grünwald Giemsa ×400)



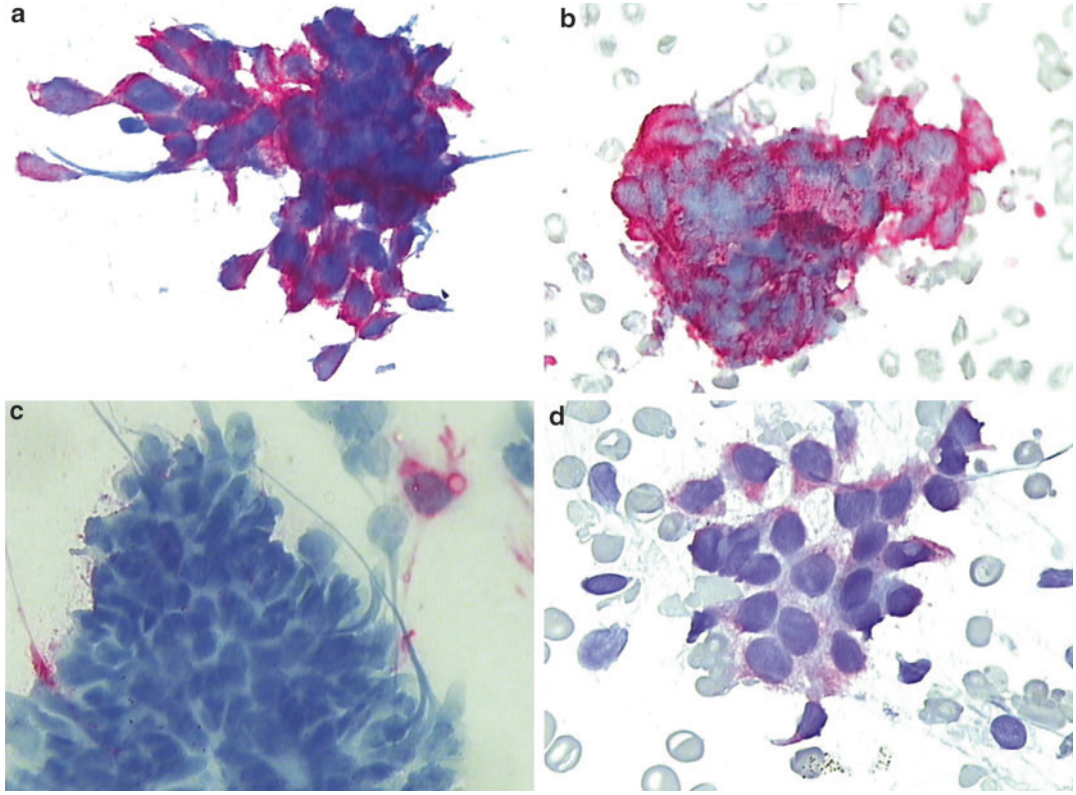
Penile Neoplasias, Cytological Findings, Fig. 9 Basal cell carcinoma. (**a**, **b**) Small uniform cells with scanty cytoplasm, hyperchromatic round or oval nuclei and granular chromatin (Dermal swab of the penis, May-Grünwald Giemsa ×1000)

***Pathology*:** Paget's disease was described by Paget in 1874 as mammary and extramammary. Extramammary Paget's disease is considered an adenocarcinoma originating from the skin or skin appendages in areas with apocrine glands. The primary location is the vulvar area, perianal region, scrotum, penis, and axillae as an erythematous plaque of indolent growth, with well-defined edges, fine scaling, excoriations, exulcerations, and lichenification (Lopes Filho et al. 2015).

***Cytology findings*:** With the imprint smears, various sizes of Paget's cells were evident on a monolayer sheet but there was no overlapping. Their cell borders were defined, and their cytoplasma were abundant. The shape of the nuclei was mostly round or oval, and the nuclear rims were thin and irregular.

The chromatin patterns were finely granular or reticular (Sheue-mei et al. 1986) (Fig. 11).

***Differential diagnosis*:** Paget's disease is a rare disease which can mimic various types of



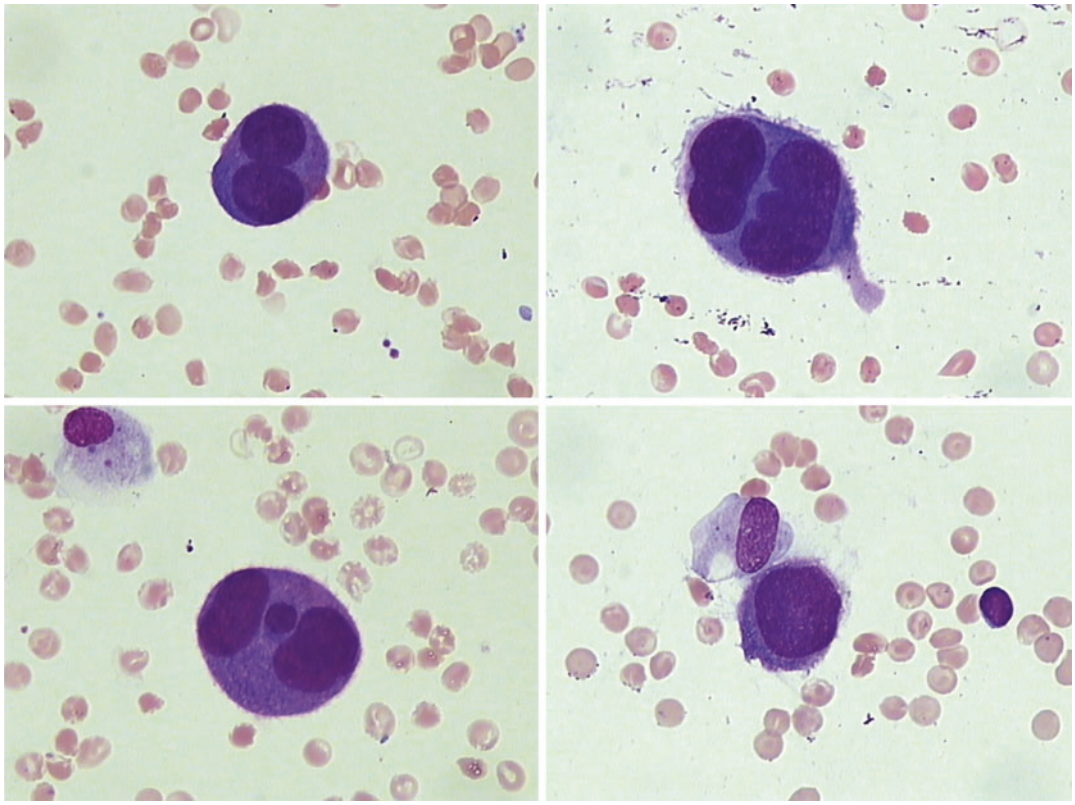
Penile Neoplasias, Cytological Findings, Fig. 10 Basal cell carcinoma. Immunocytochemistry. (**a**, **b**) Pancytokeratin positive cells, (**c**) cells negative for vimentin, (**d**) weak positivity to CK5/6 (LSAB ×1000)

dermatosis. Differential diagnosis includes the following dermatoses: Bowen's disease, tinea cruris, contact dermatitis, lichen simplex, lichen planus, psoriasis, and seborrheic dermatitis. The Paget's cells are larger than keratinocytes, have clear chromatin, a prominent nucleolus, and gray–blue cytoplasm, and they may appear vacuolated. All these characteristics of the Paget's disease unlike various dermatoses are consistent with their glandular nature. A high index of suspicion is required, combined with biopsy and immunohistochemical staining in order to make the correct diagnosis.

Melanoma

***Definition*:** Malignant melanoma is a malignancy of melanocytes. Penile melanoma is a rare cancer with a poorer prognosis than that of cutaneous melanoma as there is usually a delay in the presentation and diagnosis (Van Geel et al. 2007). The incidence of primary penile melanoma is thought to be between 0.1% and 0.2% of all melanomas, and the disease constitutes less than 2% of all malignancies at this site. Melanoma of the glans penis may be epidermal or mucosal in origin. Mucosal melanoma arises from the distal urethra while epidermal melanomas arise from the glans (Sanchez-Ortiz et al. 2005; Tritton et al. 2008).

***Pathology*:** Penile melanomas usually presents as a pigmented macule, papule, or ulceration with an irregular border; however, it can also be unpigmented. It is typically found on the glans penis and less often on the prepuce. The American Joint Committee on Cancer system for classifying cutaneous melanomas includes the depth of

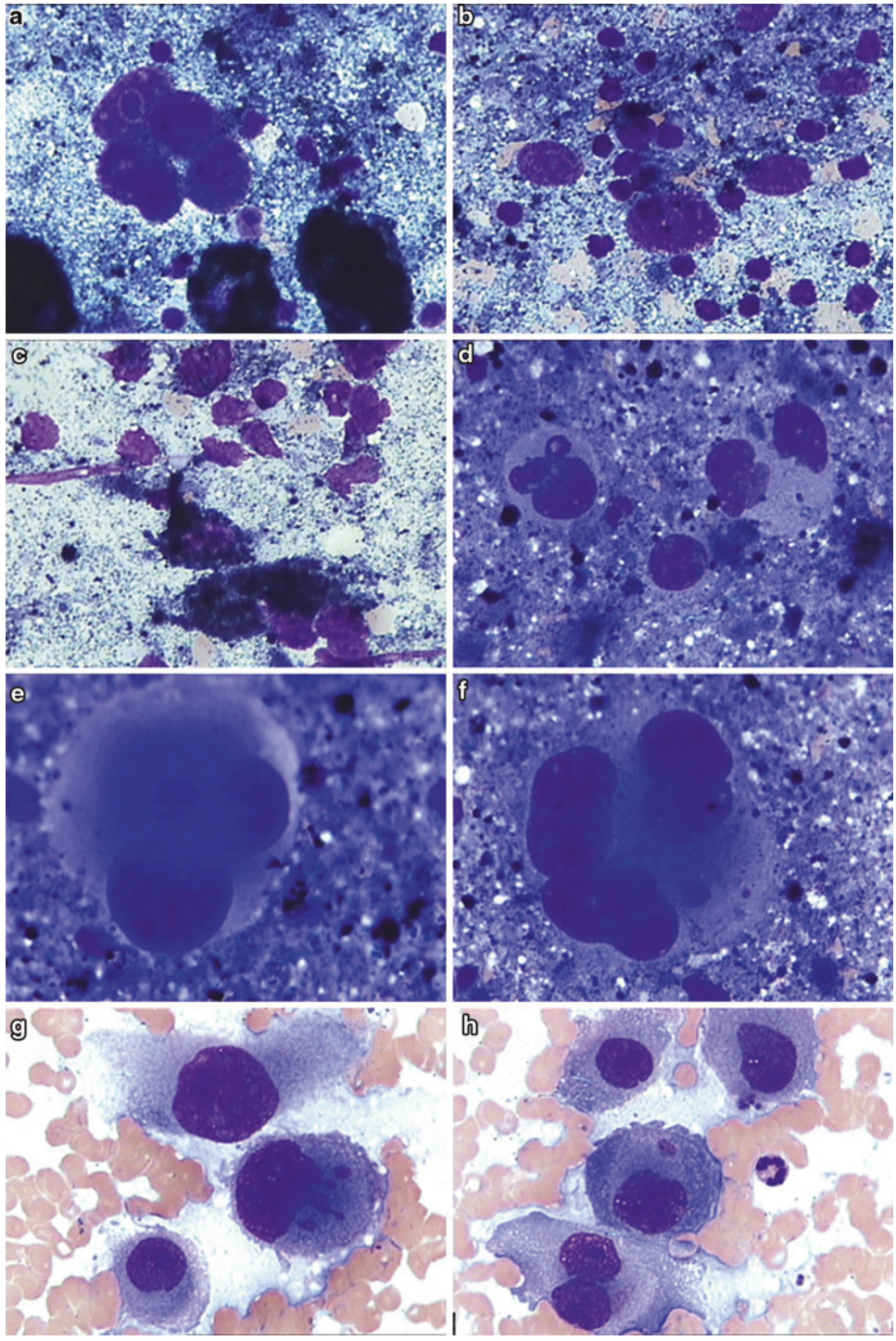


Penile Neoplasias, Cytological Findings, Fig. 11 Adenocarcinoma – Paget disease. Large Paget's cells with defined borders, abundant cytoplasm, round or oval nuclei, and irregular nuclear rims (Dermal swab of eczematosed lesion of the penis, May-Grünwald Giemsa ×1000)

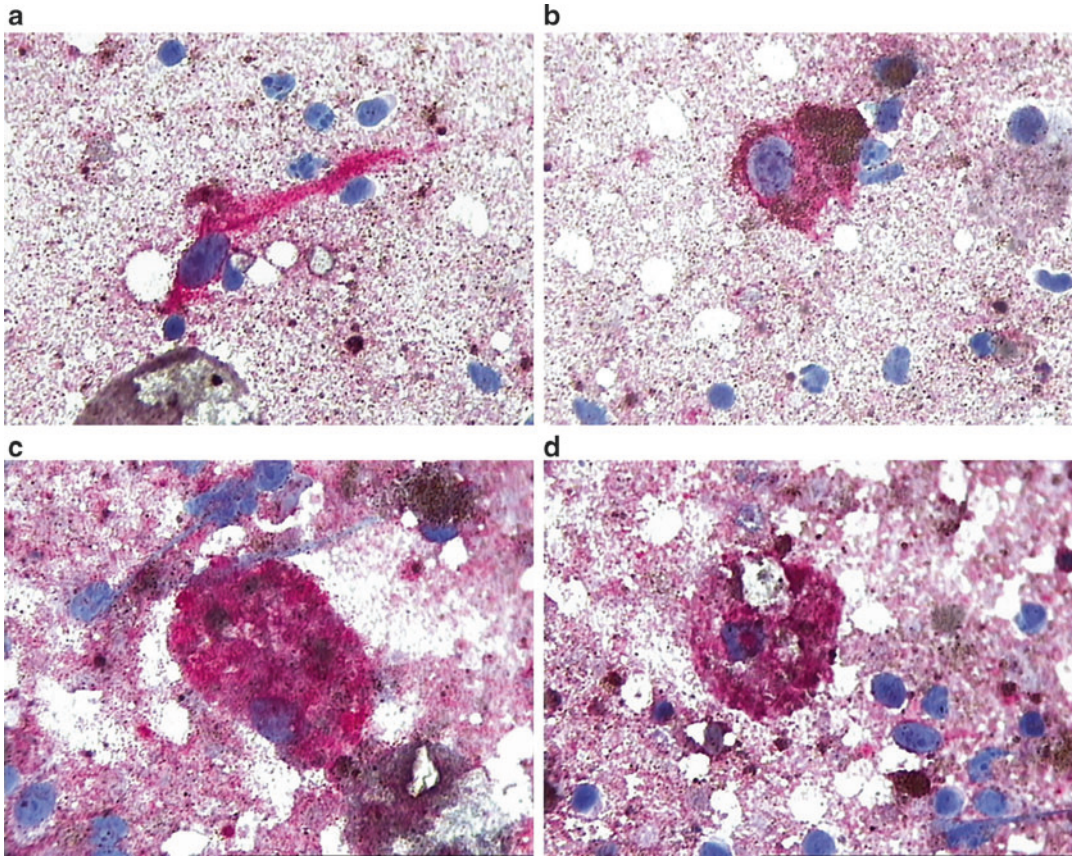
invasion (Clark staging) and tumor thickness (Breslow level, direct measurement). Hematogenous metastases occur through the vascular structures of the corporal bodies; lymphatic spread to the regional lymphatic ilioinguinal nodes occurs by lymphatic permeation (Pettaway et al. 2007). Histopathological examination of specimens is performed based on the cellular differentiation, pleomorphism, solidity, and grade of tumor according to WHO's schema.

***Cytology findings*:** Malignant cells from melanoma (epitheliod and pleomorphic subtype) usually contain moderate amounts of cytoplasm, and cytoplasmic melanin pigment is identified in tumor cells. Melanin pigment may also be seen in macrophages. The cells exhibit moderate to marked nuclear pleomorphism and, in some cases, contain binucleate and multinucleate cells. Nuclear chromatin is finely or coarsely granular, and nuclear membrane irregularities may be seen. Nucleoli are often prominent, and some cells may contain large eosinophilic macronucleoli. Intranuclear cytoplasmic invaginations (pseudoinclusions) may be seen (Fig. 12). Sometimes the background contains necrotic debris (Murali et al. 2008). Additionally, diagnosis is supported by immunostainings with a specific marker for melanoma (Protein S-100 +, HMB – 45 +, Melan A) (Fig. 13).

***Differential diagnosis*:** Differential diagnosis includes: dysplastic nevi, pigmented basalioma, pigmented Bowens disease, and seborrheic keratoses. Malignant cells from melanoma (epitheliod and pleomorphic subtype) usually contain



Penile Neoplasias, Cytological Findings, Fig. 12 Melanoma. (**a**–**f**) Various size of atypical mono- and multinucleated cells with large lobulated nuclei, prominent nucleoli, moderate amounts of cytoplasm with melanin pigment in tumor cells, macrophages, and extracellular. (**g**, **h**) Amelanotic pleomorphic cells (Fine-needle aspirate of inguinal lymph node, metastatic melanoma of the penis, May-Grünwald Giemsa ×1000)



Penile Neoplasias, Cytological Findings, Fig. 13 Melanoma. Immunocytochemistry. (**a**, **b**) Malignant cells immunostained for HMB45, (**c**, **d**) cells positive for Melan A (Fine-needle aspirate of inguinal lymph node, metastatic melanoma of the penis, LSAB ×1000)

moderate amounts of cytoplasm, and cytoplasmic melanin pigment is identified in tumor cells.

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Pericardial Effusions, Cytological Findings

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Synonyms

Cardiac tamponade; Pericardial fluid; Pericardiocentesis fluid

Definition

The pericardial sac is a double-walled serosal membrane that surrounds the heart and lies in

the middle mediastinum. The pericardial cavity is the space lying between the parietal and visceral layers of the pericardial sac. Normally, it contains a small amount of fluid that allows the heart to move without friction. A pericardial effusion (PE) results from the accumulation of fluid within that cavity. When the heart is compressed by a rapid accumulation of large amount of fluid, the effusion is called "cardiac tamponade" and can be life threatening and requires immediate intervention and pericardiocentesis (Kabukcu et al. 2004).

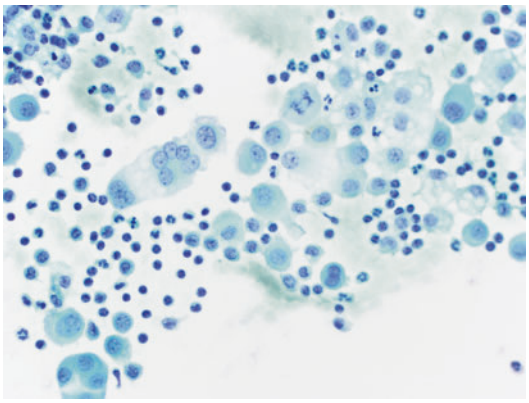
The causes of PE vary depending on the geographic location and type of hospital. Benign PEs are caused by uremia, viral and bacterial pericarditis, connective tissue diseases, and trauma (Figs. 1–3). In geographic areas with high incidence of tuberculosis, tuberculous pericarditis is the most common cause reported particularly causing recurrent PEs (Shahbaz Sarwar and Fatimi 2007). In a tertiary care hospital, malignancy is the most common cause. Cancer-related PE was reported to be associated with adverse outcome and those cytologically malignant had worse prognosis. The most common causes of malignant effusions are lung, breast, lymphoma/leukemia, and mesothelioma. Idiopathic PE constitutes up to 20% of reported effusion (García-Riego et al. 2001; Perreira et al. 2006).

Macroscopy

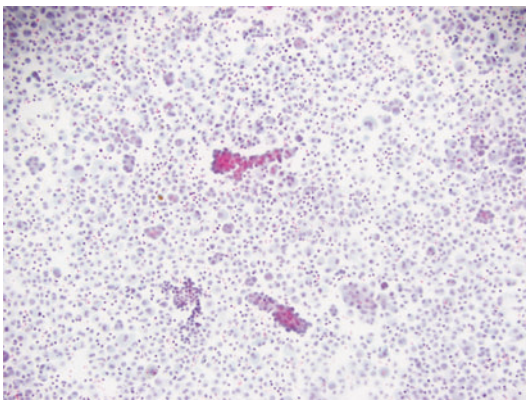
Gross appearance varies depending on the underlying etiology. Hemopericardial tamponade is deep red, similar in appearance to blood.

Microscopy

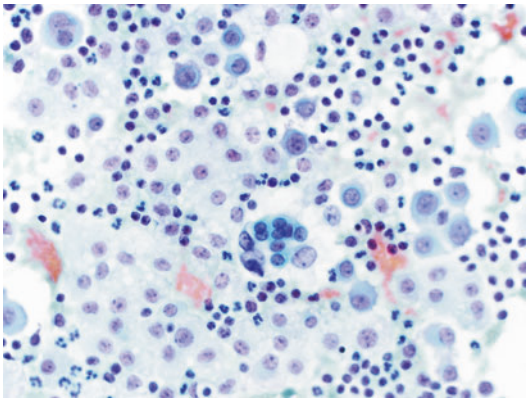
Cytological features of a PE will depend on whether it is an exudate or transudate. The fluid



Pericardial Effusions, Cytological Findings, Fig. 2 High power, Papanicolaou stain demonstrating reactive pericardial fluid from a patient with pericarditis. Notice the enlarged mesothelial cells, multinucleation, and frequent mitotic figures (Pap stain)



Pericardial Effusions, Cytological Findings, Fig. 1 Low power, Papanicolaou stain demonstrating highly cellular pericardial fluid with numerous papillary groups from a patient with long-standing heart failure (Pap stain)



Pericardial Effusions, Cytological Findings, Fig. 3 High power, Papanicolaou stain demonstrating a different area from the same patient. Notice the cluster of cells in the center with nuclear enlargement and hyperchromasia (Pap stain)

constituents are similar to those described in pleural and peritoneal fluids. While not officially published based on a study, it is a known fact among practicing cytopathology experts that reactive mesothelium in PE can look significantly more atypical than their counterpart in pleural and peritoneal effusion. It is postulated that this atypia may be the result of the constant heart beating and consequently more irritation to the pericardial serosal surface.

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Peritoneal Effusions, Cytology

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Synonyms

Ascites; Paracentesis

Definition

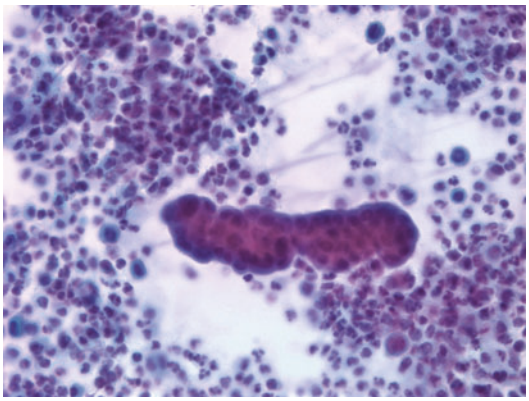
As with all serous cavities, the peritoneal cavity is lined by a flat layer of mesothelial cells and normally contains a small amount of serous fluid. When this fluid accumulates in pathologic quantities, it is referred to as an effusion and, in the case of the peritoneal cavity, ascites. The accumulated fluid can be divided clinically into transudates and exudates. Transudates are an ultrafiltrate of plasma resulting from increased hydrostatic pressure and/or decreased oncotic pressure. In the abdominal cavity, the most common cause of ascites is decreased oncotic pressure associated with cirrhosis. Transudates have low lactate dehydrogenase (LDH) levels and low total protein concentration. They are almost always associated with a benign effusion. Exudates result from damage to the mesothelium and are associated with elevated LDH levels and total protein concentration. Exudates are typically associated with malignant and inflammatory processes.

Macroscopy

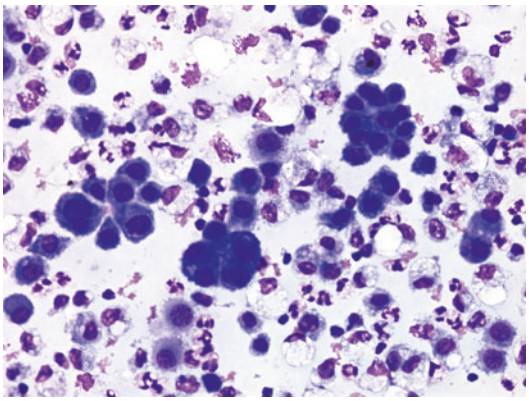
The gross appearance will vary from clear to turbid or bloody depending on the underlying etiology.

Microscopy

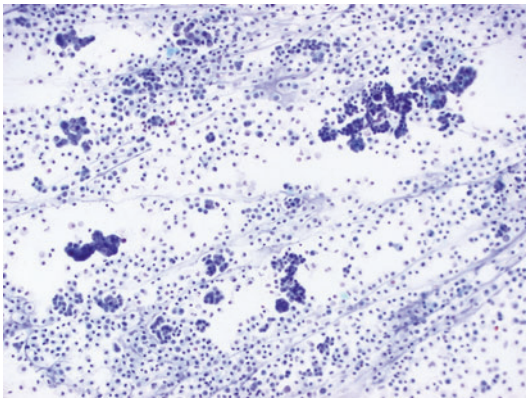
The main cellular constituent of transudative peritoneal effusions is mesothelial cells, the lining cells of the peritoneal cavity, occurring in small numbers along with histiocytes and few inflammatory cells. Exudative peritoneal effusions also contain mesothelial cells, often with reactive features, as well as other cell types (Cibas and Ducatman 2009; Demay 2012). Reactive mesothelium occurs in a variety of conditions most commonly related to either infection (Fig. 1) or long-standing disease. Liver cirrhosis (Fig. 2) and peritoneal dialysis (Fig. 3) are the most common causes that can produce striking mesothelial hyperplasia sometimes with atypia (Yousef and Michael 2010; Han et al. 1998). Other cell types can include malignant cells derived from mesothelial cells (mesothelioma) or other malignant



Peritoneal Effusions, Cytology, Fig. 1 Medium power, Papanicolaou stain demonstrating reactive mesothelial cells in a background of acute inflammation in a patient with acute bacterial peritonitis (Pap stain)



Peritoneal Effusions, Cytology, Fig. 3 Medium power, Diff-Quik stain demonstrating reactive clusters of mesothelium in a background of acute inflammation from a patient presenting with chronic renal failure and peritoneal dialysis (Diff-Quik stain)



Peritoneal Effusions, Cytology, Fig. 2 Low power, Papanicolaou stain demonstrating hyperplastic mesothelium with numerous clusters and branching papillary groups in a background of mixed inflammation from a patient presenting with liver cirrhosis and ascites (Pap stain)

neoplasms. In males, the most common malignant primary sites are the GI tract, pancreas, prostate, and hematopoietic malignancies in descending order. In females, the most common malignant primary sites are ovary, breast, uterus, GI tract, and hematopoietic malignancies in descending order (Pereira et al. 2006).

Differential Diagnosis

The differential diagnosis of these specimens depends on the cellular elements present in the effusion. A common differential diagnosis includes reactive mesothelial cells, mesothelioma, and adenocarcinoma. In a specimen in which lymphocytes predominate, the differential diagnosis can include reactive lymphocytosis and lymphoma.

P

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Peritoneal Washings (PW)

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Synonyms

Culdocentesis; Gutter wash; Pelvic wash

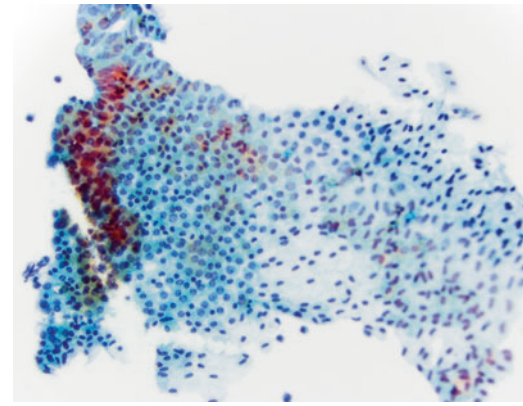
Definition

After evacuating the peritoneal cavity of any pre-existing fluid, washings are obtained by instilling sterile saline or other physiologic solution into the peritoneal cavity, and then evacuating the fluid. The evacuated fluid is then heparinized and submitted for cytologic examination. The most common indication for obtaining peritoneal washings is for staging of gynecologic malignancies.

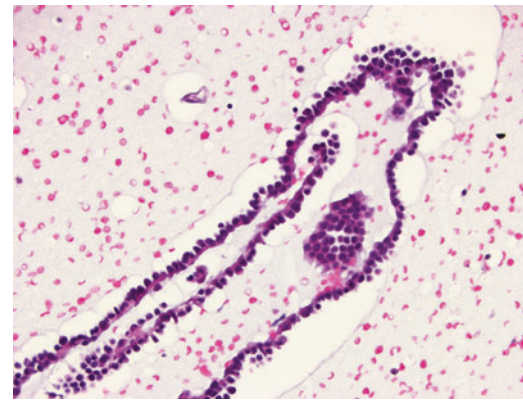
Microscopy

Normal PW typically contains sheets of benign mesothelial cells. These sheets are seen on the smears as monolayer sheets of cells with cobblestone appearance (Fig. 1). Because these cells are forcefully removed during washing with normal saline, the nuclei may exhibit slight atypia or degenerative changes and wrinkling of the nuclear membranes. These features should not be mistaken for malignancy. On cell blocks, these mesothelial sheets frequently are embedded perpendicularly and appear as thin strips of cells sometimes with scalloped contour on one side (Fig. 2). Other constituents that may be present are histiocytes, skeletal muscle, and adipose tissue. Inflammatory cells including lymphocytes and neutrophils can be seen in a variety of inflammatory conditions.

Benign findings reported in PW include collagen balls, Müllerian inclusions/endosalpingiosis,

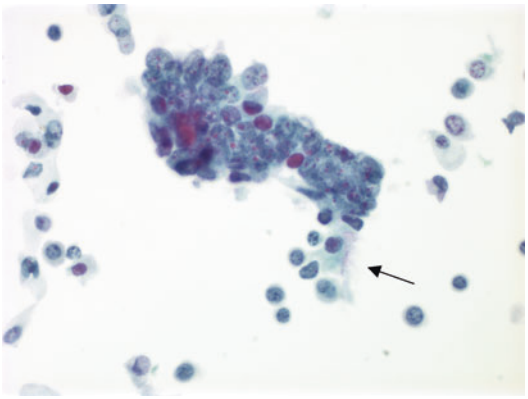


Peritoneal Washings (PW), Fig. 1 Medium power, Papanicolaou stain demonstrating a sheet of benign mesothelium as seen in peritoneal washing. Notice the cobblestone arrangement, mild nuclear variation (Pap stain)

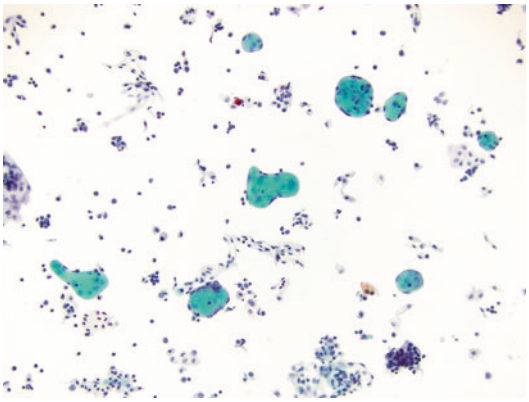


Peritoneal Washings (PW), Fig. 2 Medium power, H&E stain demonstrating strips of benign mesothelium as seen in cell blocks (H&E stain)

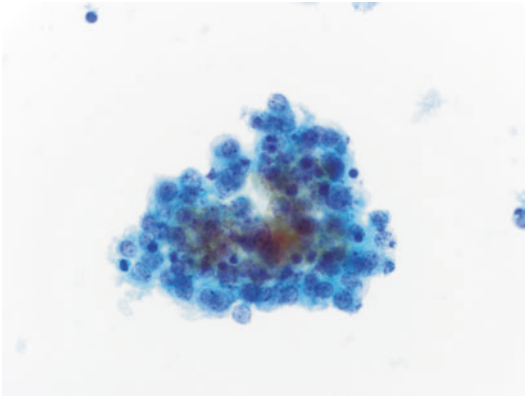
shed ciliated tubal cells, detached ciliary tufts, and psammoma bodies. Benign tubal type epithelium can be seen as a result of shedding while manipulating the tubes or in endosalpingiosis (Fig. 3). Benign endometrial cells appearing as small bland cells in cohesive clusters and hemosiderin-laden macrophages can be seen in endometriosis (Fig. 4) (Ventura et al. 2005). Collagen balls (Fig. 5) were reported to occur in up to 4.5% of peritoneal washes. These are collagen fragments covered by flattened mesothelial cells, correlate with minute papillary stromal projections on the ovarian surfaces, and have no clinical or



Peritoneal Washings (PW), Fig. 3 High power, Papanicolaou stain demonstrating a fragment of ciliated tubal cells (*arrow*) shed during surgical manipulation (Pap stain)



Peritoneal Washings (PW), Fig. 5 Low power, Papanicolaou stain demonstrating collagen balls in a peritoneal wash from a patient undergoing hysterectomy for a large uterine fibroid (Pap stain)



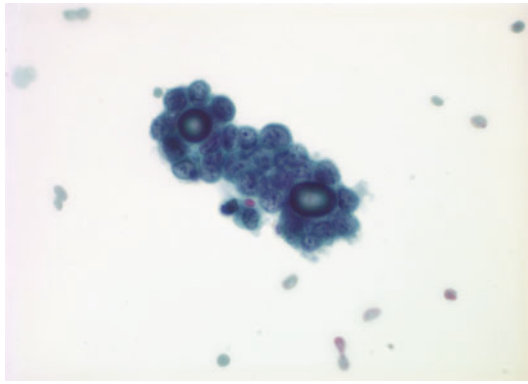
Peritoneal Washings (PW), Fig. 4 High power, Papanicolaou stain demonstrating a cluster of benign endometrial cells in a peritoneal wash from a patient with biopsy-confirmed endometriosis (Pap stain)

diagnostic significance (Wojcik and Naylor 1992). Müllerian inclusions/endosalpingiosis present as tubular or papillary structures with a central psammoma body surrounded by a single layer of cuboidal cells often displaying slight atypia. Mesothelial hyperplasia presenting as papillary clusters may also be seen particularly in cases with a large mass that posed constant irritation of the mesothelial surface.

Malignant conditions that may be identified in peritoneal washings are described elsewhere and include gynecologic malignancies of ovarian, tubal, endometrial, and cervical origins. Gastrointestinal and pancreaticobiliary carcinomas and primary peritoneal mesothelioma can also less commonly be identified.

Differential Diagnosis

The most common differential diagnosis in peritoneal washings is between reactive mesothelial cells and malignancy, most commonly adenocarcinomas of gynecologic origin. Diagnostic pitfalls include the distinction of reactive mesothelium, endosalpingiosis, and endometriosis from low-grade carcinomas (Fig. 6) (Ravinsky 1986). In a study by Ziselman et al., the following criteria were reported as essential to an accurate evaluation: (1) cells suspicious for malignancy should be present both as clusters and as single cells and should be morphologically similar to the correlating surgical resection, (2) the patient must have a known history of malignancy that resembles the current malignant cells, and (3) the suspicious cells must be morphologically different from the background reactive mesothelium (Ziselman et al. 1984). Such differential can now be easily resolved with the advent of immunochemistry performed on a well-prepared cell block.



Peritoneal Washings (PW), Fig. 6 High power, Papanicolaou stain demonstrating a cluster exhibiting cells with subtle cytologic atypia surrounding a psammoma body from a patient with borderline serous tumor of the serosal surface (Pap stain)

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Pheochromocytoma, Cytological Findings

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Synonyms

Chromaffinoma; Malignant paraganglioma; Phaeochromoblastoma

Definition

Pheochromocytoma is a neuroendocrine tumor of the medulla of the adrenal glands (World Health Organization Classification – 2004). Extra-adrenal tumors have origin in the sympathetic, parasympathetic, or in the chromaffin tissue that is present after birth and are called as paraganglioma.

Prevalence is poorly defined.

Clinical Features

Most pheochromocytomas secrete mainly norepinephrine.

Clinical symptoms are the result of an excessive catecholamine secretion and of individual catecholamine sensitivity. A percentage of the patients can be asymptomatic.

Familial forms and cystic pheochromocytomas are generally asymptomatic mainly because these familial forms secrete essentially epinephrine as does the normal adrenal medulla. In cystic forms the reason might be due to the fact that large cystic tumors release, most frequently, metabolized catecholamines, showing low urine catecholamine concentrations (Karagiannis et al. 2007; John et al. 1999).

Main symptoms are:

Elevated blood pressure with

- Paroxystic hypertension in 48% of the patients (variable duration and frequency)
- Headache and altered mental status
- Intracerebral hemorrhage
- Diaphoreses
- Palpitations
- Anxiety

Cardiovascular symptoms like

- Shock
- Myocarditis
- Arrhythmias
- Dilated cardiomyopathy
- Edema
- Heart failure

Curiously most typical clinical symptoms occur in association to benign tumors.

A patient with a typical clinical context of pheochromocytomas presents a classic triad of constitutional symptoms that include periods characterized by headaches, palpitations, and diaphoresis in association with severe hypertension. These four characteristics, when presented together, are strongly suggestive of pheochromocytomas.

Biochemical diagnosis should be done using a combination of different tests such as 24-h urine assays of catecholamines, vanillylmandelic acid, total and fractionated metanephrines and their metabolites (useful in cystic tumor, for the above-mentioned reason), as well as plasma concentration assay of metanephrines. Generally pheochromocytomas secreting more premature catecholamines are more prone to develop metastases (Gao et al. 2008). A ratio between epinephrine and epinephrine plus norepinephrine can be used as an indication of tumor differentiation.

Magnetic resonance imaging (MRI) should be used as the first-line method of image documentation. Metaiodobenzylguanidine (MIBG) scintigraphy should be used for confirmation although it has a sensitivity of about 80–85%. This low sensitivity occurs mainly in malignant tumors (Hoegerle et al. 2002). Dopa positron emission tomography (PET) or dopamine-PET seems to be the method of choice (Kudva et al. 1999).

- **Incidence**
  Pheochromocytoma is a rare disease which affects less than 200,000 people in the US population (http://rarediseases.info.nih.gov/).
- **Age**
  Hereditary tumors occur earlier (5–69 years) than sporadic tumors (4–81years).
  Approximately 10% occur in children.
- **Sex**
  Pheochromocytomas show equal frequency in males and females.
- **Site**
  Over 90% of pheochromocytomas/paragangliomas are located within the adrenal gland and 95% in the abdomen (Thompson 2002). Extra-adrenal pheochromocytomas/paragangliomas develop more frequently in the para-aortic region, along the sympathetic nervous system, in the para-axial region or in the connective tissue adjacent to bladder and pelvic organs.
  Sporadic tumors are more frequently unilateral whether familiar tumors are frequently bilateral. In general 10% are bilateral. Multifocal pheochromocytomas occur most often in MEN 2 followed by VHL, PGL 1, and PGL 4 associated tumors.
- **Treatment**
  Treatment of pheochromocytomas can be a challenge due to the high variability of clinical settings. Surgical resection of the tumor is the treatment of choice and aggressive excision of metastases should also be performed. In cases where surgical approach is not possible, palliative care should be taken into account in order to minimize the symptoms and pain. In tumors with MIBG uptake, radiation with [131] MIBG can be an option.
- **Outcome**
  Approximately 10% of pheochromocytomas are malignant and about 10% have metastases at presentation. Malignant tumors can have long survivals, varying between months to decades. Metastasis can appear many years after the first diagnosis.
  Prognostic factors include tumor size and stage at diagnosis. However, the only reliable criterion for malignancy is the presence of metastasis. DNA ploidy favors benignity, while tetraploid or aneuploid tumors are more frequently malignant. Malignancy is rare in tumors associated with (**n**euro**f**ibromatosis) NF1 and (**m**ultiple **e**ndocrine **n**eoplasia) MEN2; however, in tumors associated with **s**uccinate **de**h**y**d**r**ogenase (SDHB) gene mutations, malignancy occurs in **a** high percentage of cases.

Macroscopy

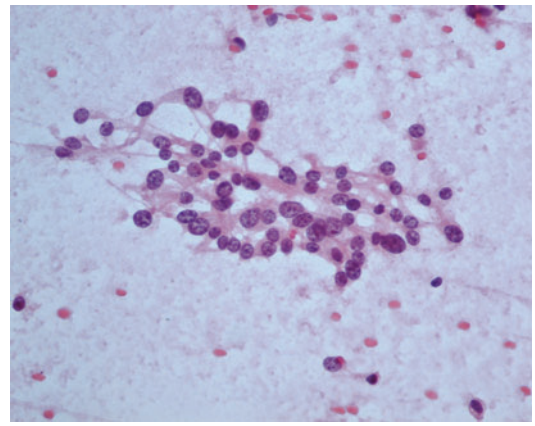
Sporadic tumors measure between 3 and 5 cm and weigh an average of 100 g. Cut surface is gray/

white and darkens with exposure to air. Necrosis, cystic degeneration, and hemorrhage can be present but they do not correlate with malignancy.

Microscopy

On histology, cells anastomose in trabecular cords, nests with a "zellballen" (German expression meaning balls of cells) typical pattern, surrounded by sustentacular cells. Solid areas can predominate. The cells are large and polygonal, and cytoplasm can vary from pale to granular and from basophilic to eosinophilic or even amphophilic. The nuclei are eccentric with variable pleomorphism showing nuclear pseudo-inclusions and intracytoplasmic hyaline globules. Spindle cells or ganglion-like cells can be present. In order to help in distinguishing benign from malignant tumors, some authors have advanced a series of pathologic criteria (PASS system), in analogy with the approach used in adrenal cortical tumors (Weiss system) (Geisinger et al. 2004). However, this PASS system is not completely unfailing and till now no reliable histological criteria can distinguish a malignant from a benign tumor. The new challenging approach in order to discriminate these two entities has been represented by the application of molecular markers. Many factors like endothelial growth factor, endothelial receptor B, secretogranin II, and telomerase activity have been studied but yet in the research field.

The use of fine needle aspiration cytology, when pheochromocytomas are suspected, is not of common use in many centers mainly due to the suspected risk of a sudden hypertension crisis. Cytological smears are characterized by neoplastic cells disposed single or in loose clusters. The cells vary in size, shape, and pleomorphism (Fig. 1). Nuclear pleomorphism can be absent so that the tumor may resemble a carcinoid. Bizarre nuclei or nuclear multilobulation and pseudo-inclusions can be seen with multinucleations. Cytoplasm is large but poorly defined with frayed borders and with a red granularity in Giemsa stains (Gimenez-Roqueplo et al. 2003).



Pheochromocytoma, Cytological Findings, Fig. 1 Scraped cytology from an adrenal pheochromocytoma in an 11-year-old girl – Cytological smears are characterized by neoplastic cells in loose clusters and in this case with mild pleomorphism. Nuclear chromatin is coarse with salt and pepper-like appearance. In this case cytoplasm is poorly defined, forming elongated fibrillar extensions and simulating fibrillary neuroblastoma stroma (H&E stain)

Immunophenotype

Pheochromocytomas are immunoreactive for chromogranin, synaptophysin, **n**on**s**pecific **e**sterase (NSE) and **c**luster **d**ifferentiation-CD56. Sustentacular cells are positive for (subunit alpha) S100 protein. Variable expression for vimentin, Bcl2, and (**h**uman **m**elanoma **b**lack) HMB45 has been reported for neoplastic cells. No expression is usually found with MelanA/Mart-1, inhibin, keratin (usually), **e**pithelial **m**embrane **a**ntigen (EMA), and calretinin. Intracytoplasmic hyaline globules are PAS + diastase resistant.

Molecular Features

Most cases are sporadic even if 10% of the cases are associated to syndromes like neurofibromatosis (0.1–5.7% of the cases with NF1), Von Hippel-Lindau (in 20% of the cases of VHL), MEN1, MEN2, or inserted in the pheochromocytomas/paraganglioma syndrome (PGL) (mutation of succinate dehydrogenase gene) 1. PGL syndrome represents about 20% of apparent sporadic cases. To date there are four recognized mutations of the **SDH** gene: type 1

associated with mutations of D subunit (SDHD), type 2 (gene unknown), type 3 associated with mutations of subunit C (SDHC), and type 4 associated with mutations of subunit B (SDHB) (Walther et al. 1999; Neumann et al. 2005).

Differential Diagnosis

The heterogeneity of cytological pheochromocytoma patterns raises multiple misdiagnoses namely with epithelial and mesenchymal tumors which include adenocarcinoma, renal cell carcinoma, adrenal cortical tumors, and neuroendocrine tumors. Cytology should be always evaluated in the contest of clinical setting. Immunostains are also essential in the differential diagnosis. Pheocromocytomas stain positively for neuroendocrine markers and negative for keratins in general, while most of the other tumors that are referred as potential pitfalls, express keratins.

Renal cell carcinoma (RCC) is morphologically difficult to distinguish, from pheochromocytoma. Neoplastic cells of a RCC lesion are less pleomorphic with central nuclei and typically round eosinophilic nucleoli. Cell borders are well defined in general and cells have intracytoplasmic glycogen. Unlike RCC, pheochromocytomas are negative for CD10 and EMA.

Adrenocortical tumors although positive in most cases to synaptophysin are negative for chromogranin, whether pheochromocytomas are positive for both markers.

Rarely pheochromocytomas have lipofuscin or melanin-like pigment raising diagnostic problems with melanoma. Unlike melanoma pheochromocytomas are negative for Melan A.

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Phyllodes Tumor, Cytological Findings

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Synonyms

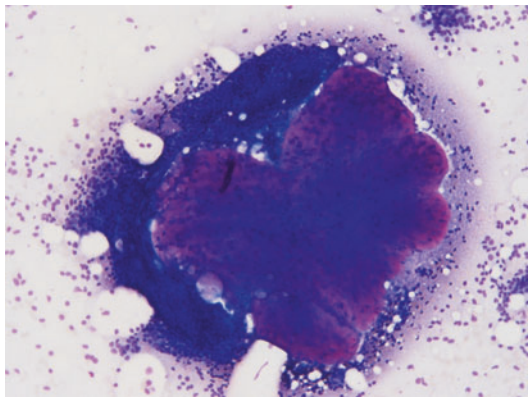
Cystosarcoma phyllodes

Definition

This entity is defined as a true biphasic tumor composed of both epithelial and cellular stromal components (Fig. 1).

Clinical Features

- **Incidence**
  These are rare tumors which comprise less than 1% of all breast tumors and have an incidence of about 2.1 per million.
- **Age**
  In Western countries, the average age is 45 years old about 20 years older than patients with fibroadenomas. The tumor is rarely found in adolescents and the elderly. Malignant phyllodes tumors (PTs) develop on average 2–5 years later than benign PTs.
- **Sex**
  More frequent in women.
- **Site**
  There is not a specific site on the breast.
- **Treatment**
  Hence the phyllodes tumor (PT) can be extremely difficult to differentiate from a fibroadenoma, which is sometimes treated with a nonoperative approach. For this reason, early diagnosis of phyllodes tumor is crucial so that the correct management of the tumor, which often does include surgery, can be pursued as early as possible. Surgical management of PT has also been a source of debate over the years. Some authors have argued for simple mastectomy for PT because of the risk of local recurrence after more conservative procedures. However, studies have shown no differences between breast-conserving surgery versus mastectomy in terms of metastasis-free survival or overall survival in malignant PTs, despite the higher incidence of local recurrence that comes with breast-conserving surgery.
- **Outcome**
  Controversial opinion has been reported concerning the diagnostic and prognostic histologic parameters that are used for classifying benign or malignant PT. Approximately 12% of PT recurs.



Phyllodes Tumor, Cytological Findings, Fig. 1 Phyllodes tumor. Note the lobulated cellular stroma surrounded by epithelial cells (Giemsa staining)

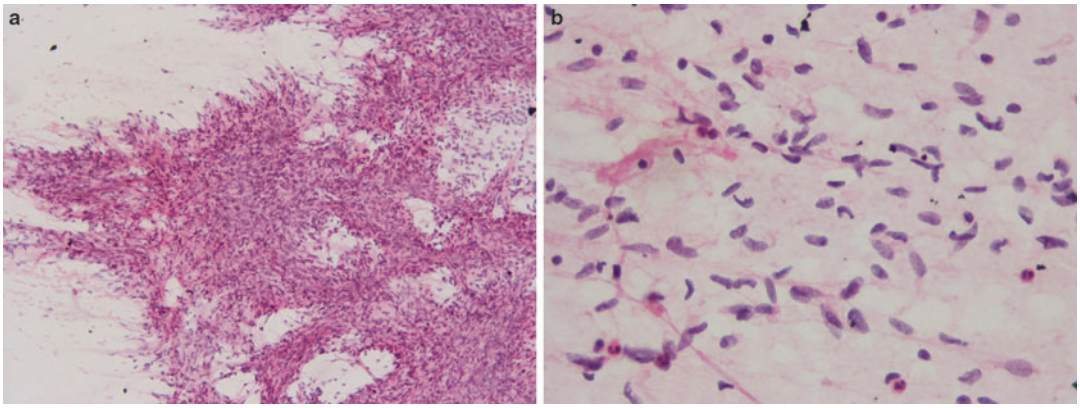
Macroscopy

This lesion is macroscopically defined by a large nodular lump measuring greater than 4 cm in diameter maximum. Twenty percent of tumors grow larger than 10 cm. The lesion is usually firm, mobile, well defined, round, macrolobulated, and painless.

Microscopy/Cytology

Cytological distinction with fibroadenoma is based on assessment of the cellularity of the stroma component. The key features for the diagnosis of PT are (1) cellular smears and (2) the presence of biphasic population of epithelial and hypercellular stromal component characterized by spindle-shaped cells. The evidence of atypical stromal component should be linked with a malignant PT instead of a benign PT. Bipolar and spindle naked nuclei can be present.

Histologically, PTs are divided into benign, borderline, and malignant histotypes based on the microscopic appearance of the stromal component. Approximately 15–30% of all cases are classified as malignant. Histologic appearance may not, however, correlate with clinical



Phyllodes Tumor, Cytological Findings, Fig. 2 (**a**) Phyllodes tumor. Note high cellular spindle cell stroma in a case of malignant phyllodes tumor (HE staining). (**b**) Phyllodes tumor. Note isolated spindle cell naked nuclei (HE staining)

behavior, as both malignant and borderline tumors have been shown to be capable of metastasizing. Pathologic factors associated with poor prognosis include greater than five mitotic figures per high power field, infiltrating margins, severe atypia, stromal overgrowth, stromal component other than fibromyxoid, and tumor necrosis (Fig. 2).

Immunophenotype

Antibodies to vimentin, desmin, actin, high- and low-molecular-weight keratins, and S100 protein were used for immunohistochemical staining. CD34 is frequently positive in benign PT. In contrast, CD117 (CKIT) is more frequently expressed in malignant PT.

Molecular Features

Loss of nuclear beta catenin, amplification of MYC, aberrant expression of P53, and deletion of 9p21 (CDKN2A) are described in malignant PT.

Differential Diagnosis

The major important differential diagnosis is fibroadenoma and the distinction between benign and malignant PTs. Cellularity of the stroma is the most important cytological criteria to distinguish fibroadenoma from PT. When this differential is not possible, our option is to diagnose a fibroepithelial tumor and ask for excision. The presence of prominent epithelial component with some hyperplastic features may lead to a false-positive diagnosis of breast cancer for which the presence of hypercellular stroma and abundant naked nuclei might be helpful.

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Plasmacytomas, Cytological Findings

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Synonyms

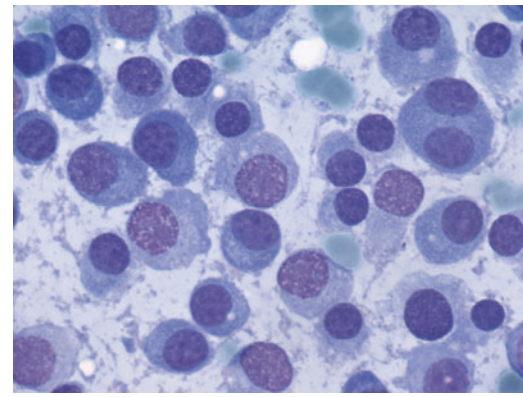
Extramedullary plasmacytoma; Extraosseous plasmacytoma

Definition

Extraosseous (extramedullary) plasmacytomas are localized plasma cell neoplasms that arise in tissues other than bone.

Clinical Features

- **Incidence**
  3–5% of all plasma cell neoplasms.
- **Age**
  Median age at diagnosis is 55 year.
- **Sex**
  2/3 are male.
- **Site**
  Majority (80%) of extraosseous plasmacytomas occur in the upper respiratory tract including the oropharynx, nasopharynx, sinus, and larynx. In 15% of plasma cells, neoplasms occur in lymph nodes, gastrointestinal tract, bladder, CNS, breast, thyroid, testis, and parotid.
- **Treatment**
  Local radiation therapy
- **Outcome**
  25% develop regional recurrences. Progression to plasma cell myeloma is infrequent (15%), and 70% of the patients remain disease-free at 10 years.



Plasmacytomas, Cytological Findings, Fig. 1 Plasmacytoma: A typical plasma cell with eccentric round nuclei with coarse chromatin and basophilic cytoplasm. MGG

Microscopy

Fine needle aspiration cytology consists of atypical plasma cells in various stages of maturation with eccentric round or oval nuclei with basophilic cytoplasm (Fig. 1).

Immunophenotype

Monotypic cytoplasmic immunoglobulin. Usually express CD38, CD138, CD79a, and nearly always CD19 negative. Plasma cells may aberrantly express CD56 (70–80%), CD117, CD20, CD52, and CD10.

Molecular Features

Immunoglobulin heavy and light chain genes are clonally rearranged.

Differential Diagnosis

Plasmacytic lymphoma, plasmacytic hyperplasia, myeloma, metastasis of carcinoma, and melanoma

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Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings

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Synonyms

Mixed tumor

Definition

Benign tumor of myoepithelial cells

Clinical Features

- **Incidence**
  Most frequent tumor of the lachrymal glands (50–60%)
- **Age**
  Middle-aged patients
- **Sex**
  Incidence rate slightly higher for males than for females
- **Site**
  Upper outer orbital cantus
- **Treatment**
  Surgery
- **Outcome**
  Favorable

Macroscopy

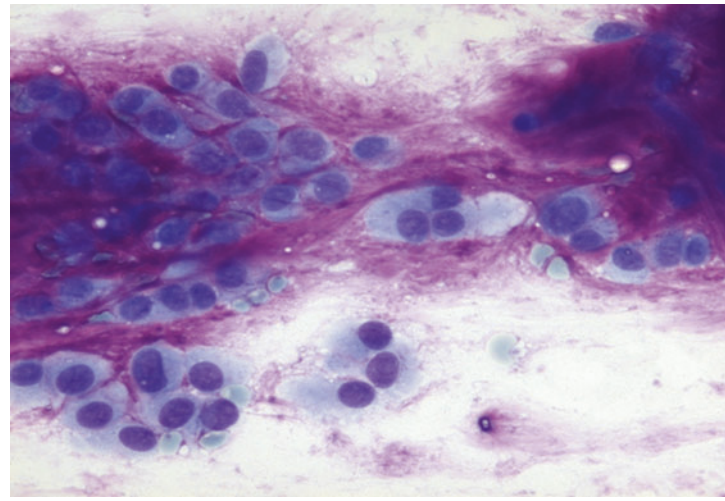
White-grayish nodule, often encapsulated

Microscopy

Pleomorphic adenoma is characterized by plasmacytoid or spindle myoepithelial cells and metachromatic matrix. These myoepithelial cells generally show eccentric nuclei and well-defined cytoplasm (Fig. 1). Cytological presentation is quite variable depending on the variable amount of the different components. In some cases, myoepithelial plasmacytoid cells are predominant, and differential diagnosis with metastases or other salivary tumors may be indicated.

Differential Diagnosis

Monomorphic adenoma, adenoid cystic carcinoma, and carcinoma ex pleomorphic adenoma



Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings, Fig. 1 FNC of a pleomorphic adenoma of lacrimal gland. Myoepithelial cells with well-defined cytoplasm and peripheral nuclei are interspersed in abundant reddish metachromatic matrix (Diff-Quik stain 270×)

Cross-References

- ▶ Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- ▶ Conjunctiva Cytology, General Aspects
- ▶ Conjunctival Inflammatory Lesions, Cytological Findings
- ▶ Conjunctival Lymphoma, Cytological Findings
- ▶ Conjunctival Melanocytic Tumors, Cytological Findings
- ▶ Conjunctival Papilloma, Cytological Findings
- ▶ Conjunctival Squamous Cell Carcinoma, Cytological Findings
- ▶ Cornea Cytology
- ▶ Cytology of the Orbit and Ocular Adnexa
- ▶ Eyelids Cytology, General Aspects
- ▶ Lacrimal Gland Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Meningioma, Cytological Findings
- ▶ Orbit Cytology, General Aspects
- ▶ Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- ▶ Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Pleomorphic Sarcomas, Cytological Findings

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Variants

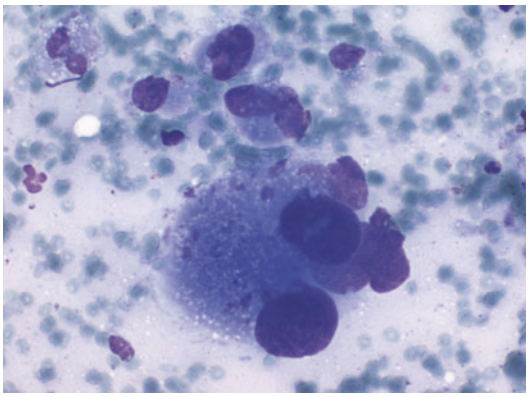
Pleomorphic malignant fibrous histiocytoma (MFH), Pleomorphic rhabdomyosarcoma, Pleomorphic malignant peripheral nerve sheet tumor, Pleomorphic liposarcoma, Pleomorphic leiomyosarcoma.

Definition

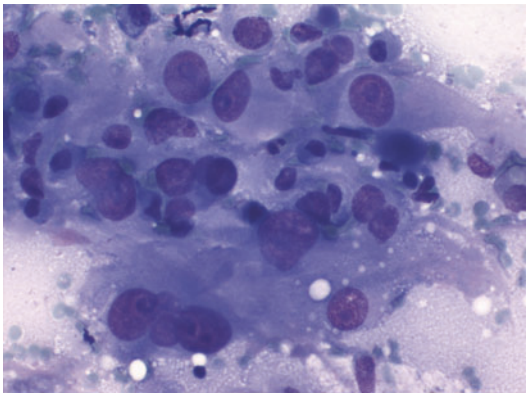
High-grade tumors arising from cells of various lineage.

Clinical Features

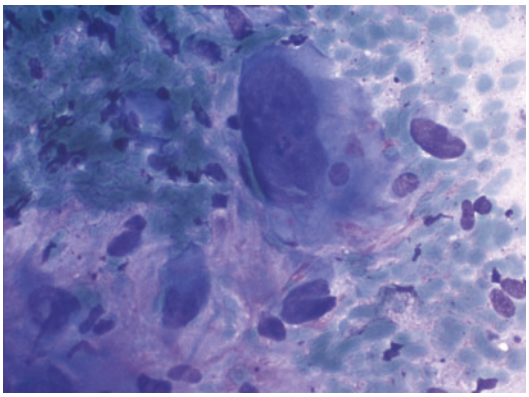
- **Incidence**
  Relatively rare sarcomas except for pleomorphic malignant fibrous histiocytoma which is the most common sarcoma (10/1,000,000) in elderly.
- **Age**
  A peak incidence around 70 years but tumors of myogenic or nerve sheet origin can be seen in young adults and even children.



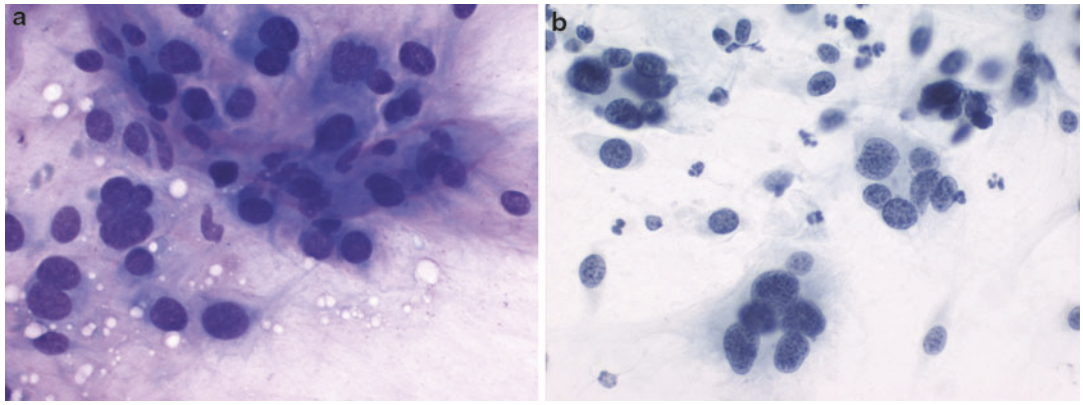
Pleomorphic Sarcomas, Cytological Findings, Fig. 1 Pleomorphic MFH: tumor cells with pleomorphic hyperchromatic nuclei and varying amount of cytoplasm. MGG



Pleomorphic Sarcomas, Cytological Findings, Fig. 2 Pleomorphic rhabdomyosarcoma: large tumor cells with nuclei of varying size and shape. Typically, many cells have a rich *gray-blue* elongated cytoplasm. MGG



Pleomorphic Sarcomas, Cytological Findings, Fig. 3 Pleomorphic MPNST: markedly pleomorphic tumor cells with poorly preserved cytoplasm. MGG



Pleomorphic Sarcomas, Cytological Findings, Fig. 4 Pleomorphic liposarcoma: highly atypical cells, some with multiple nuclei and diffuse cytoplasm in a slightly myxoid background. (**a**) MGG (**b**) Papanicolaou

- **Sex**
  A slight overrepresentation for men except for retroperitoneal leiomyosarcoma which is more common in women.
- **Site**
  Most cases in the deep soft tissue of the limbs. Pleomorphic leiomyosarcoma also in the retroperitoneum.
- **Treatment**
  Surgery, radiation, and chemotherapy.
- **Outcome**
  These are high-grade tumors with poor overall survival.

Microscopy

The tumor cells are pleomorphic with bizarre tumor giant cells often mixed with spindle cells. Cells of myogenic differentiation may have a rich eosinophilic cytoplasm. Necrosis and inflammatory cells are common (Figs. 1–5).

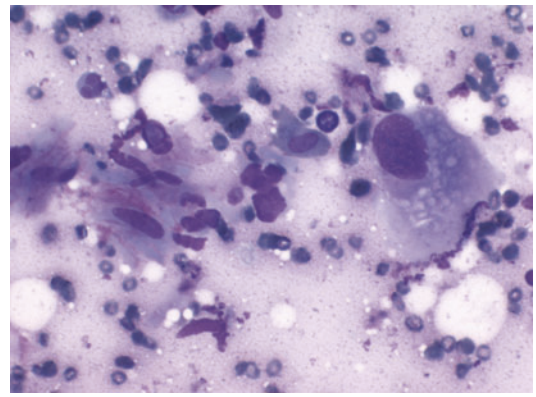
Immunophenotype

All tumors express vimentin. Malignant fibrous histiocytomas have no specific markers.

Rhabdomyosarcoma express myoglobulin, desmin, and sometimes actin.

Leiomyosarcomas are positive for desmin, SM actin, and caldesmon.

Liposarcomas are often S-100 positive.



Pleomorphic Sarcomas, Cytological Findings, Fig. 5 Pleomorphic leiomyosarcoma: large atypical tumor cell with irregular oval nuclei and spindle-shaped cells with elongated nuclei. MGG

Nerve sheet tumors are often S-100 and glial fibrillary acidic protein (GFAP) positive.

Molecular Features

No consistent structural or numerical aberrations have been reported.

Differential Diagnosis

Various pleomorphic sarcomas, pleomorphic lipoma, melanoma, sarcomatoid carcinomas, anaplastic large cell lymphoma.

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Pleural Effusions, Cytology

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Synonyms

Pleural effusion; Pleural fluid; Thoracentesis fluid

Definition

Pleural effusion is the accumulation of fluid in the pleural space. The pleural space lies between the parietal and visceral pleura and contains no more than 20 μm of fluid (0.1–0.2 mL/Kg) that is glycoprotein rich and acts as a lubricant for the movement of the lung against the chest wall during respiration.

Accumulation of pleural fluid is not a normal finding and is always considered pathologic. Since most fluids that accumulate are derived from the lung through the visceral pleura and are absorbed mainly through the parietal pleural fluid, diseases induce pleural effusion through either excess production or decrease absorption of the fluid.

Pleural effusions may also occur in concurrence with abdominal diseases especially when the lymphatic drainage across the diaphragm is compromised.

Heart failure and pneumonia are the most common causes of benign or reactive pleural effusions followed by ▶ tuberculosis (Porcel 2010). Among the abdominal diseases causing pleural effusion, ascites and pancreatitis are the most common. Both entities tend to cause unilateral effusions than bilateral. Ascites is associated with right effusion while peritonitis causes left-sided effusion. Hydrothorax has also been reported to occur with peritoneal dialysis (Lew 2010).

Macroscopy

Gross appearance of pleural fluids varies depending on the underlying etiology and cellular contents. Transudative pleural fluids are usually clear yellow while infected exudative effusions are turbid yellow or white. Hemorrhagic effusions associated with malignancy or injury tends to be dark red.

Microscopy

The microscopic findings in pleural fluids will depend on the underlying cause. Transudates, as in the case of patients with heart failure, tend to be low in cellularity and consist predominantly of histiocytes, few inflammatory cells, and few quiescent small mesothelial cells.

Exudative effusions are usually cellular and may have high leukocyte and histiocyte count. The type of leukocyte may have diagnostic implications. A high neutrophils count usually implies infection although it can occur in other systemic diseases such as lupus. High lymphocytic count raises the differential of

▶ tuberculosis or malignancy. A high basophilic count has been described to occur most commonly with benign diseases and rarely with malignancy (Okimoto et al. 2003). An ▶ eosinophilic effusion can underlie both benign and malignant disorders.

Carbon laden macrophages (anthracotic pigment) in pleural effusions have been reported to occur in crack smokers. One report suggested that they could also occur without crack abuse and when present may imply a subclinical pneumothorax (Pantanowitz et al. 2009).

Mesothelial cell hyperplasia cells can vary in number, and in some conditions such as pneumonia, liver cirrhosis or dialysis, the mesothelium may become hyperplastic and florid to the point that differentiation from adenocarcinoma NOS and Adenocarcinoma, lung and *mesothelioma* is warranted. Despite the cellularity, these reactive cells maintain a low nuclear to cytoplasmic ratio, appear monotonous with little variation in size, and do not exhibit nuclear abnormality.

The most common primary malignancies causing malignant pleural effusion in males are lung, lymphoma/leukemia, gastrointestinal tract, and genitourinary tract in descending order. In females, the most common primary sites are breast, genital tract, lung, lymphoma/leukemia, and gastrointestinal tract in descending order (Perreira et al. 2006).

In malignant effusions, there are usually two cell populations: the malignant cells and the background benign mesothelium. Malignant cells especially carcinomas frequently display high pleomorphism with chromatin clumping, irregular nuclear membranes, and prominent nucleoli. The nuclear to cytoplasmic ratio varies depending on the type of carcinoma but generally is high. Carcinoma can present as tight cell cluster, papillae, or single cells. Regardless of the pattern, evidence of dyscohesion and single abnormal cells can be detected (Michael 2012).

Malignant mesothelioma can cause a particular challenge as it displays subtle. Its differential diagnosis includes both reactive mesothelium and adenocarcinoma. Effusions with malignant mesothelioma are almost always unilateral and seldom bilateral while the other two differentials can be either unilateral or bilateral. Mesothelioma is usually highly cellular and displays a monotonous population of cells that exhibit a low nuclear to cytoplasmic ration and subtle nuclear atypia. A helpful finding is the presence of cells with wide variation of size that range from that of normal mesothelium to that of giant and frequently multinucleated cells (Michael 2012).

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Pneumocystis jirovecii, Cytological Findings

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Synonyms

Formerly known as *Pneumocystis carinii*

Definition

Pneumocystis jirovecii is a ubiquitous non-filamentous fungus and the cause of a life-threatening opportunistic pneumonia in

immunocompromised patients. *Pneumocystis* can colonize the respiratory tract of healthy individuals. Reactivation of latent *Pneumocystis* infection and de novo airborne person-to-person transmission have both been suggested as possible mechanisms causing *Pneumocystis* pneumonia (PCP) in immunocompromised patients.

Pneumocystis cannot be cultured in vitro and the gold standard for PCP diagnosis is direct microscopic visualization of the fungus in bronchoalveolar lavage (BAL) fluid by immunofluorescence or Grocott methenamine silver stain.

Clinical Features

- **Incidence**
  PCP remains one of the most common AIDS-defining diseases in HIV-infected patients. With combined antiretroviral therapy and chemoprophylaxis, the incidence has significantly declined to approximately 4 cases per 1,000 person-years. In contrast PCP is increasing in non-HIV-infected immunosuppressed patients, including patients with long-term corticosteroid therapy, TNFα inhibitors, and collagen vascular disease.
- **Age**
  PCP can affect immunocompromised patients at any age.
- **Sex**
  A male predominance has been reported.
- **Site**
  Pneumocystis jirovecii affects the lung. Extrapulmonary infections are rare and most commonly occur in hilar lymph nodes, bone marrow, liver, and spleen.
- **Treatment**
  Trimethoprim-sulfamethoxazole is most commonly used for prophylaxis and treatment of PCP.
- **Outcome**
  Non-HIV-infected immunosuppressed patients have a more acute clinical course and a higher PCP mortality rate than HIV-infected patients. The mortality rate is 30–60% compared to 10% in HIV-infected patients. A high level of suspicion for PCP in immunocompromised patients allows for early diagnosis and treatment and is crucial to reduce mortality.

Macroscopy

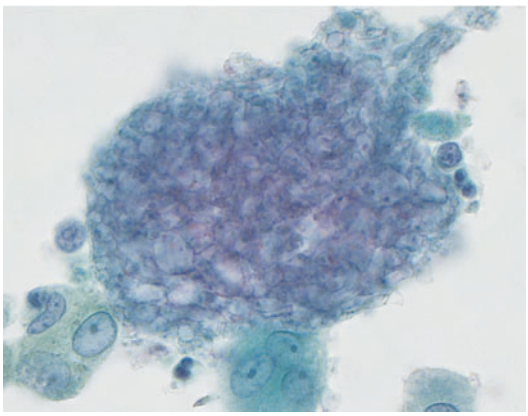
Details on how to process BAL fluid are described in the entries on BAL.

Microscopy

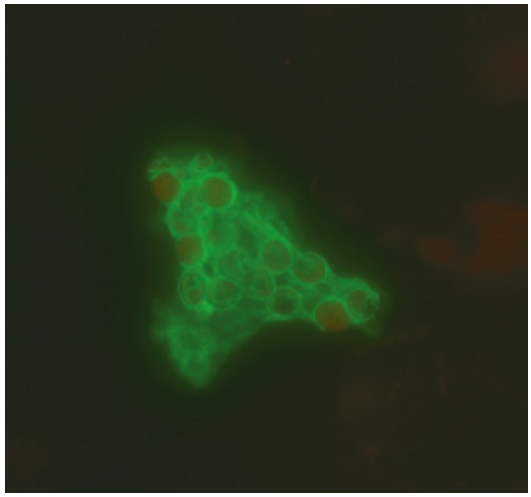
Pneumocystis shows two major development forms, the trophic (1–5 μm) and the cystic (~5–8 μm). In PCP the trophic form predominates and represents 90% of all development forms.

The classic morphological pattern of PCP is a foamy intra-alveolar exudate with only mild or even absent inflammation. The exudate contains abundant trophic forms admixed with cysts of *Pneumocystis*.

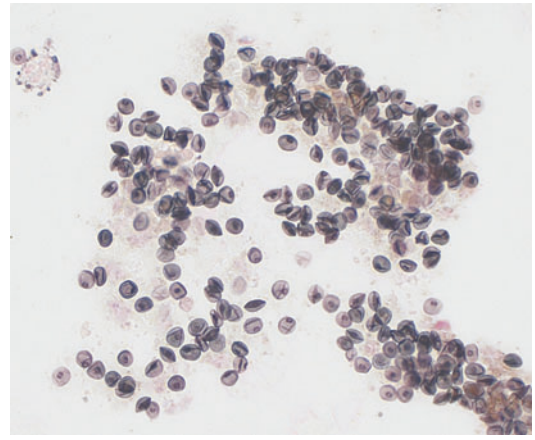
Papanicolaou (PAP) does not stain the trophic forms and barely stains the cyst walls. The aggregates of cysts are indirectly visible in the exudate as small empty spaces (Fig. 1). Up to 50% of non-HIV and 20% of HIV-infected patients with PCP lack the classic alveolar exudate due to a low *Pneumocystis* load, and therefore, the infection



***Pneumocystis jirovecii*, Cytological Findings, Fig. 1** Foamy alveolar exudate with empty spaces. The empty spaces contain the cyst forms of *Pneumocystis*, which are barely stained by Papanicolaou (Bronchoalveolar lavage, Papanicolaou stain, original magnification ×600)



***Pneumocystis jirovecii*, Cytological Findings, Fig. 2** Bright apple-green reaction of *Pneumocystis* with the Monofluo™ *Pneumocystis jirovecii* immunofluorescence test kit (Bio-Rad Laboratories, Montreal, Canada) (Bronchoalveolar lavage, original magnification ×600)



***Pneumocystis jirovecii*, Cytological Findings, Fig. 3** The morphology of the round to oval and cup-shaped cysts with the *dark dot*-like density is diagnostic for *Pneumocystis* (Bronchoalveolar lavage, GMS, original magnification ×600)

can easily be missed on PAP-stained slides. The nuclei of all development forms can be visualized by Romanowsky stains (Giemsa, Diff-Quick), but the staining is often weak and unspecific. Immunofluorescence or fungal stains like Grocott methenamine silver (GMS) should therefore be performed if PCP is suspected. Especially patients with PCP after solid or hematopoietic stem cell transplantation can have very few organisms and a fast and sensitive detection is crucial for timely treatment and prognosis.

Detection of *Pneumocystis* by immunofluorescence has the highest diagnostic sensitivity and specificity. Fluorescence conjugates monoclonal anti-*Pneumocystis*-antibodies detect all development forms, and the evaluation is easy and fast due to the bright apple-green fluorescence (Fig. 2).

Grocott methenamine silver (GMS) stains the cyst walls and shows a morphology, which is diagnostic for *Pneumocystis*: The cysts are dark brown, round to oval, and cup shaped or crescent when collapsed. They have a characteristic central or peripheral dot-like density due to a local thickening of the cyst wall (Fig. 3).

Molecular Features

PCR-based assays are highly sensitive for the detection of *Pneumocystis* DNA in respiratory specimens but are less specific compared to direct microscopic visualization of the organism. A positive PCR result does not necessarily correlate with disease and can detect *Pneumocystis* DNA in asymptomatic colonization. Quantitative real-time PCR assays show promising results, but the cutoff, at which the fungal load differentiates patients with PCP from patients with colonization, needs to be validated.

Differential Diagnosis

The differential diagnosis of *Pneumocystis* includes yeasts like *Histoplasma capsulatum*, *Candida* species, and *Cryptococcus neoformans*. *Pneumocystis* lacks budding and has the characteristic central dark dot-like density, which is not observed in other fungi with yeast-like morphology.

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Pneumonia, Effusions Associated with

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Synonyms

Parapneumonic effusions; Simple or complicated parapneumonic effusions

Definition

A pleural effusion associated with pneumonia is referred to as "parapneumonic effusion." The majority of parapneumonic effusions are not infected "simple effusions." However, up to 40% of the effusion are infected "complicated effusions" or frank pus "empyema" (Murali et al. 2010; Wrightson and Davies 2010). The pleural surface particularly the parietal pleura overlying the mediastinum is highly susceptible to infection due to its close opposition to other organs. Meanwhile, the parietal surface overlying the diaphragm is more resistant to infection. The infected pleura usually undergo acute necrotizing pleuritis and responds to the infection with a high protein exudative effusion.

Clinical Features

- **Incidence**
  The incidence of parapneumonic effusions in adult population ranges between 20–57% of all pleural effusions (Light 2006; Wrightson and Davies 2010). In the pediatric population, parapneumonic effusions were reported to contribute 70% of all pleural effusions.

  The most common infectious causes of pleural effusions are bacterial and tuberculous infections.Fungal and protozoal infections are less common.*Staphylococcus aureus*, *Streptococcus pneumoniae*, and enteric gram-negative bacilli are the most common bacterial infections involved with parapneumonic effusions. Anaerobic bacterial infections are usually associated with aspiration pneumonia, lung abscess, or empyema (Murali et al. 2010).
- **Age**
  Parapneumonic effusions can occur at any age.
- **Sex**
  Parapneumonic effusions can occur in both males and females.
- **Site**
  It is usually ipsilateral to the infected site.
- **Treatment**
  Every patient with the diagnosis of pneumonia should be worked up for the presence of effusion in order to triage treatment and avoid complications. Effective therapy usually depends on the severity and should aim to control the infection and allow re-expansion

of the lungs. Not all patients require drainage with thoracocentesis unless the fluid exceeds 10 mL on a lateral decubitus film or is symptomatic. Small effusions tend to resolve with treatment of their underlying causes (Beers and Abramo 2007). If evaluation of the fluid confirms the diagnosis of empyema based on cell count and other laboratory test, a chest tube should be placed. Pleural fluids that do not meet the criteria of empyema are divided into uncomplicated or complicated fluids based on their pH, glucose, and LDH and culture results. In patients with uncomplicated effusions, antibiotic therapy is first initiated and thoracocentesis is repeated within 12 h. If the fluid remains uncomplicated, chest tube placement is not indicated. Patients who develop parapneumonic effusion while receiving antibiotic therapy for the pneumonia are not likely to develop empyema or require drainage (Marcea and Metersky 2006). Chest tube placement is indicated in patients with complicated fluids. Up to 15% of patients with empyema will require thoracoscopy and surgical intervention.

- **Outcome**
  Simple effusions resolve with the treatment of the underlying infection without complications. Complicated effusions can be associated with severe morbidity and mortality if treatment was delayed and up to 22% of patient could die. The majority of cases respond to treatment and have long-term survival, provided they survive the first year. Complications with chronic disease are unusual with the current treatment modalities. Pleural thickening occurs in about 13% of patients and tends to have no functional significance. Rarely significant pleural fibrosis occurs and results in restriction in patient's activity (Wrightson and Davies 2010).

Macroscopy

The gross appearance of the fluid is clear in simple effusions, but is generally turbid and opaque in complicated effusions.

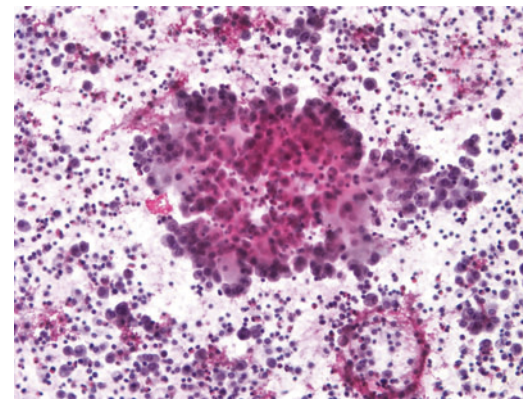
Microscopy

Simple effusions may have few mesothelial cells in a background of histiocytes and scattered neutrophils. Complicated bacterial parapneumonic effusions tend to have high cellularity with more mesothelial cells in a background of profound acute inflammatory infiltrate. Eosinophils can also be present in significant numbers (Fig. 1). Fluids from empyema tend to be pure pus. Highly proliferative reactive mesothelium is frequently encountered and in long-standing cases, numerous papillary clusters are not unusual. The mesothelium displays features recognized in reactive mesothelium such as monotony of cell size and low nuclear to cytoplasmic ratio. It is the author's experience that fluids associated with viral pneumonia can have a high lymphocytic count.

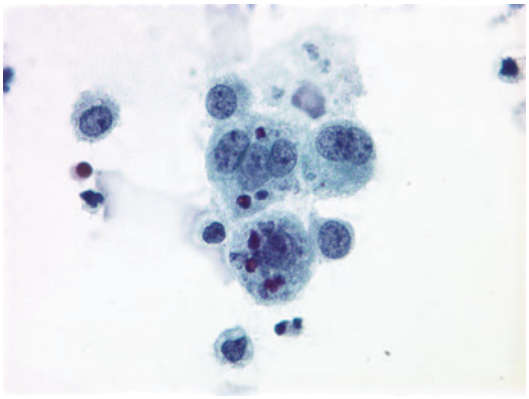
Mesothelial cells can acquire phagocytic properties and may be seen with intracytoplasmic inflammatory cells (Fig. 2).

Immunophenotype

The reactive mesothelium will react positively to desmin and negative to EMA. It will also be non-reactive to other immunostains reactive with



Pneumonia, Effusions Associated with, Fig. 1 Medium power, Papanicolaou stain demonstrating a complex papillary mesothelial group in the background of acute inflammation consisting of both neutrophils and eosinophils from a patient with bacterial pneumonia (H&E stain)



Pneumonia, Effusions Associated with, Fig. 2 High power, Papanicolaou stain demonstrating reactive mesothelium with intracytoplasmic phagocytosed inflammatory cells (Pap stain)

adenocarcinoma such as CEA, MOC-31 and Ber-Ep4, B72.3, Leu-MI (CD 15), etc.

Molecular Features

In problematic cases, molecular techniques such as assessment for chromosomal abnormalities will be negative in comparison to those described for mesothelioma and adenocarcinoma.

Differential Diagnosis

The reactive mesothelium can pose a diagnostic challenge and mimic mesothelioma and adenocarcinoma. The presence of neutrophils by itself is not sufficient to sort this differential since many of the patients with malignant effusions display high neutrophil counts particularly after a chest tube is placed. The finding should be interpreted in light of the clinical history and the pathologist should back up and consider the reactive diagnosis particularly in patients presenting in acute distress such as fever and rapidly developing shortness of breath.

Immunostains can assist in the differential diagnosis of reactive mesothelium versus adenocarcinoma and reactive mesothelioma, as described elsewhere.

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Polymorphous Low-Grade Adenocarcinoma, Cytological Findings

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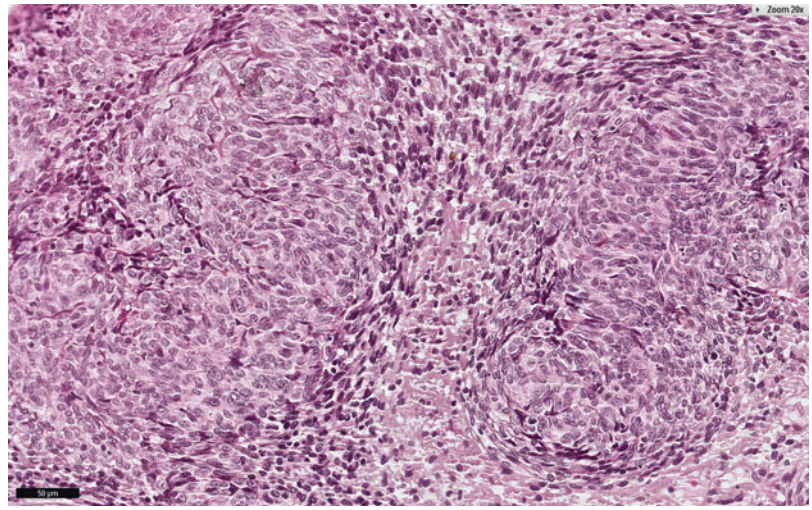
Synonyms

Lobular salivary carcinoma; Terminal duct carcinoma

Definition

Polymorphous low-grade carcinoma (PLGA) is an epithelial-derived, low-grade salivary adenocarcinoma with low metastatic potential (Barnes et al. 2005; Ellis and Auclair 2008). PLGA is composed of epithelial and myoepithelial cells with characteristic blind morphology of the nuclei. PLGA shows polymorphous patterns including cribriform, papillary, tubular, and solid growth (Fig. 1).



Polymorphous Low-Grade Adenocarcinoma, Cytological Findings, Fig. 1 Polymorphous low-grade adenocarcinoma. Palate. Solid areas of blend cells with elongated and clarified nuclei (H&E stain)

Clinical Features

- **Incidence**
  The incidence of PLGA is changing in the recent years, since many adenoid cystic carcinomas are actually diagnosed as PLGA. PLGA currently accounts for 20–25% of salivary gland carcinomas.
- **Age**
  PLGA arises after the third decade and its peak of incidence is between 60 and 80 years of age.
- **Sex**
  There is 2 to 1 female-to-male predominance.
- **Site**
  PLGA occurs almost exclusively in minor salivary glands, especially in the palate, oral mucosa, upper lip, and retromolar region. Hard palate is the site of near 60% of cases.
- **Treatment**
  Complete surgical excision is a therapeutic modality. Lymph node clearance is not necessary.
- **Outcome**
  Recurrences are rare and more frequent in tumors with palatal bone involvement.

Macroscopy

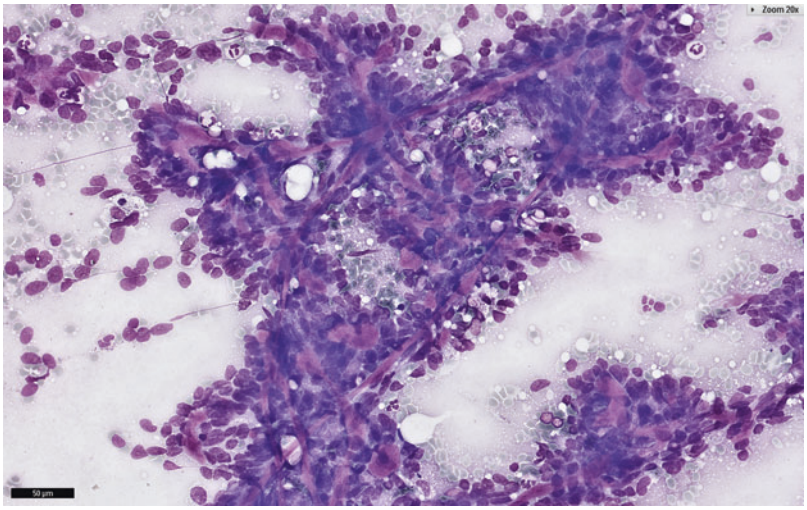
PLGA is a gray-yellow uncapsulated mass. Some large tumors may infiltrate bone structures of the palate. Rarely, surface epithelium may be ulcerated.

Microscopy

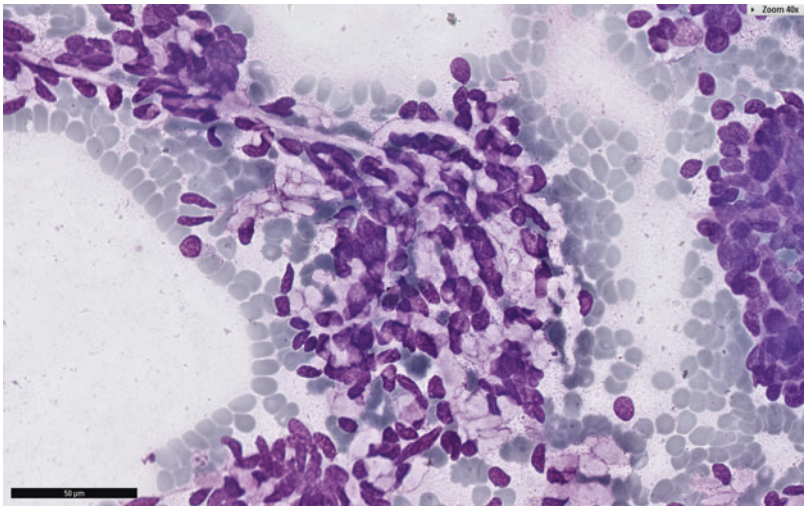
Smears in PLGA are usually hypercellular and cell-rich and stroma-rich. PLGAs belong to the group of tumors exhibiting predominant myoepithelial cell morphology (Klijanienko and Vielh 1998a).

The cellular material is composed of polyhedral or oval cells, isolated, and in clusters. Formations with pseudopapillary pattern and dendritic stromal cores are frequently observed (Fig. 2). The cytoplasm is stripped in most of the cases. Slight cytonuclear atypia may be present. Mitotic figures are usually absent. Chromatin and nuclear shape are characteristic; nuclei are usually oval and large and chromatin is dusty, making impression of "clarified" nuclei (Fig. 3). Intranuclear inclusions may be also occasionally seen. Psammoma bodies are sometimes present.

Stromal component consists of tubular structures, hyaline globules in various proportions (Fig. 4), and nonspecific connective debris (Klijanienko and Vielh 1998a).



Polymorphous Low-Grade Adenocarcinoma, Cytological Findings, Fig. 2 Polymorphous low-grade adenocarcinoma. Pseudopapillary pattern with dendritic stromal cores resembling EMEC (MGG stain)



Polymorphous Low-Grade Adenocarcinoma, Cytological Findings, Fig. 3 Polymorphous low-grade adenocarcinoma. Clear chromatin and elongated nuclei (MGG stain)

Immunophenotype

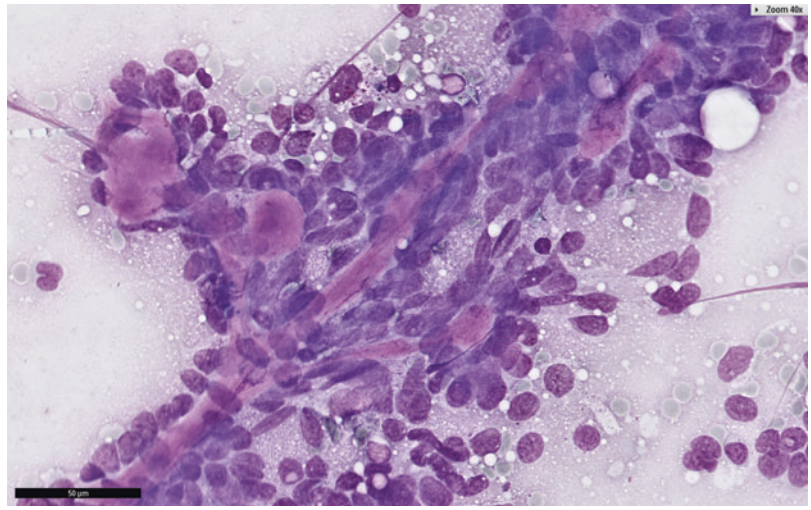
PLGA is immunoreactive for epithelial and myoepithelial markers such as SMA, cytokeratins, EMA, and CEA.

Molecular Features

The molecular events underlying the pathogenesis of PLGA are poorly understood, and no recurrent genetic aberrations have been identified so far.

Differential Diagnosis

Every tumor arising on the palate should be differentiated with PLGA. In addition, PLGA must be differentiated from other tumor entities showing myoepithelial cell predominance like adenoid cystic carcinoma (ACC), epithelial-myoepithelial carcinoma (EMEC), and cellular pleomorphic adenoma (PA). The difficulties in distinguishing PLGA from ACC are due to the occasional presence of hyaline globules. In PLGA, the nuclei are larger and the chromatin is dusty (clear), whereas the cells in ACC are smaller and darker. Moreover,



Polymorphous Low-Grade Adenocarcinoma, Cytological Findings, Fig. 4 Polymorphous low-grade adenocarcinoma. Hyaline globules similar to those from ACC (MGG stain)

occasional necrosis seen in solid types of ACC is not observed in PLGA. Similarly, papillary formations in stromal cores are unusual in ACC. Finally, PLGA arises mostly exclusively in accessory salivary glands whereas ACC arise mostly in major salivary glands (Klijanienko and Vielh 1997).

EMEC may be undistinguishable from PLGA, but it may show a bimodal cell population: ductal inner cells and clarified, external cells. This is especially evident when Papanicolaou stain is used. In both entities tubular structures and hyaline globules may be present. Once again, EMEC arises exceptionally in the minor salivary glands (Klijanienko and Vielh 1998b).

Cellular PA may be misdiagnosed when eosinophilic stromal fragments in PLGA are present. It is composed of myoepithelial cells with plasmacytoid shape. The presence of chondromyxoid stroma is strong in favor of cellular PA. Hyaline globules are exceptional in cellular PA and frequent in PLGA (Klijanienko and Vielh 1996).

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Poorly Differentiated Carcinoma of Thyroid, Cytological Findings

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Synonyms

Insular carcinoma; Poorly differentiated follicular carcinoma; Poorly differentiated papillary carcinoma

Definition

A group of tumors in an intermediate biological and clinical position in the spectrum between well-differentiated and undifferentiated carcinoma.

Clinical Features

- **Incidence**
  Incidence rates are variable with geographic variance: 15% in Northern Italy versus 1.8% in the USA. PDTC represents 2–4% of all thyroid carcinomas.
- **Age**
  Usually seen in people older than 50.
- **Sex**
  More often observed by women (sex ratio F/M 2:1).
- **Site**
  No specific site for this tumor.
- **Treatment**
  Primary treatment is usually surgery with total thyroidectomy associated with lymph nodes dissection. It may be followed by radioiodine therapy or radiotherapy or chemotherapy, the latter being essentially proposed to inoperable patients. Targeted therapy is expected.
- **Outcome**
  Prognosis is usually severe with a high risk of local recurrence and metastases.

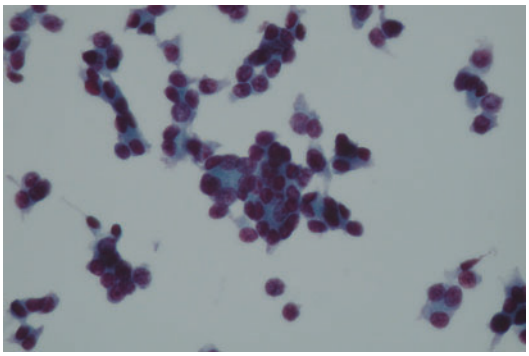
Macroscopy

This tumor is usually large, invading more or less an entire lobe of the gland; its color is gray-white with areas of necrosis.

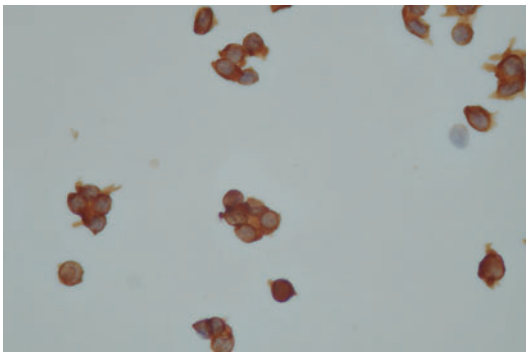
Microscopy

Fine-needle aspirations (FNA) are usually highly cellular. The most helpful criteria to suggest the diagnosis are the following (Fig. 1): (1) numerous single cells associated with cells in a trabecular or insular arrangements; (2) mitoses; (3) necrosis; (4) a high N/C ratio; (5) a relative monomorphism is usual, but nuclear polymorphism is also frequent; (6) scant cytoplasm; (7) coarse chromatin; (8) in some cases, microfollicules are visible.

Cytological criteria are quite similar on conventional smears and in liquid-based cytology.



Poorly Differentiated Carcinoma of Thyroid, Cytological Findings, Fig. 1 High cellularity on a slide with trabecular arrangement and microfollicles. The nuclei are enlarged with coarse chromatin. There is a high N/C ratio (LBC; Hologic®; Papanicolaou staining ×40)



Poorly Differentiated Carcinoma of Thyroid, Cytological Findings, Fig. 2 Cytoplasmic positivity with CK19 in all tumoral cells (LBC; Hologic®; immunocytochemistry ×40; Ventana; antibody: Novocastra b170)

Immunophenotype

Few extensive studies have been done for poorly differentiated carcinoma; a recent one shows a 100% HBME1 and Galectin-3 positivity with 80% beta-catenin positivity. Other studies have shown expression of CK19 (Fig. 2), thyroglobulin, and TTF1.

Molecular Features

Poorly differentiated thyroid carcinomas can develop de novo or represent the dedifferentiation of papillary or follicular carcinomas. p53 pathway deregulation as well as mutations in the β-catenin is a necessary step for poorly differentiated carcinoma.

Differential Diagnosis

In cases with a high cellularity, the diagnosis is usually feasible, especially when the main criteria described above are represented; the diagnosis is more difficult in cases with a suboptimal cellularity; microfollicles often lead to a diagnosis of "follicular neoplasm."

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Primitive Neuroectodermal Tumors (PNET), Cytological Findings

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Synonyms

Askin tumor; Peripheral neuroblastoma; Peripheral neuroepithelioma

Definition

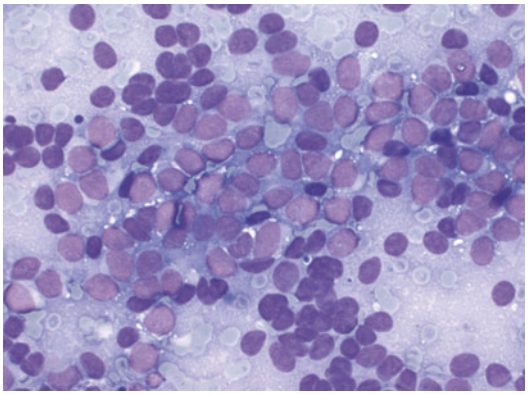
Primitive tumors with neuroectodermal differentiation.

Clinical Features

- **Incidence**
  2/1 000 000
- **Age**
  A majority of patients are below 20 years.
- **Sex**
  Males are more often affected.
- **Site**
  Diaphysis of long bones, pelvis, and ribs. Soft tissue.
- **Treatment**
  Chemotherapy, radiation, and surgery.
- **Outcome**
  Approximately 50% of the patients survive. Patients with small, peripherally located tumors have best survival.

Microscopy

Double cell population: small cells with scanty cytoplasm and dark nuclei and larger cells with



Primitive Neuroectodermal Tumors (PNET), Cytological Findings, Fig. 1 Ewing/PNET: tumor cells of two types; one with small hyperchromatic nuclei and with sparse or no cytoplasm. The second type is larger with round to oval nuclei and distinct cytoplasm with few clear vacuoles. MGG

round nucleus and rich cytoplasm often with vacuoles. Rosette structures often present.

Immunophenotype

Vimentin, CD99 positive. Synaptophysin positivity in some cases (Fig. 1).

Molecular Features

Translocation t(11;22) in 85% of cases. In some tumors t(21;22) or t(7;22).

Differential Diagnosis

Poorly differentiated synovial sarcoma, precursor lymphoma/acute lymphoblastic leukemia.

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Prostate Adenocarcinoma, Cytological Findings

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Synonyms

Acinar adenocarcinoma of the prostate

Definition

Malignant tumors arising in the glandular epithelial cells.

Clinical Features

- **Incidence**
  Varies between different ethnicities. 250/100,000 in Scandinavia.

- **Age**
  Increases with the age. Seldom seen before 40 years.
- **Sex**
  It is an exclusive male disease.
- **Treatment**
  Observation, surgery, radiation, ablation, endocrine therapy, and chemotherapy.
- **Outcome**
  Protracted course in most cases but highly aggressive tumors occur.

Microscopy

Well differentiated: slightly enlarged often crowded cells with poorly defined cytoplasm and small nucleoli forming adenomatous structures.

Moderately well differentiated: enlarged crowded overlapping cells with marked nuclear atypia and distinct nucleoli. Dense clusters of tumor cells but few adenomatous structures can be observed.

Poorly differentiated carcinoma: enlarged cells with irregular nuclei and marked nucleoli. Mostly naked nuclei but poorly formed adenomatous structures can occur. Necrosis common.

Immunophenotype

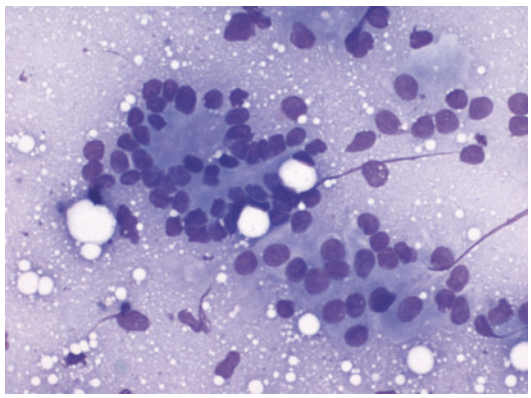
Cytokeratin, PSA, and adrogen receptor positive.

Molecular Features

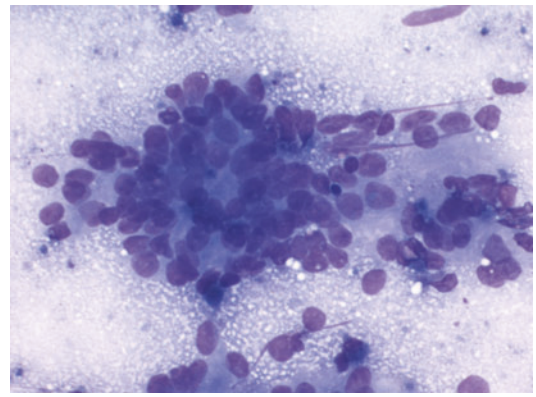
No single genetic aberration has been identified but patients with BRAC-2 germ line mutations are at an increased risk.

Differential Diagnosis

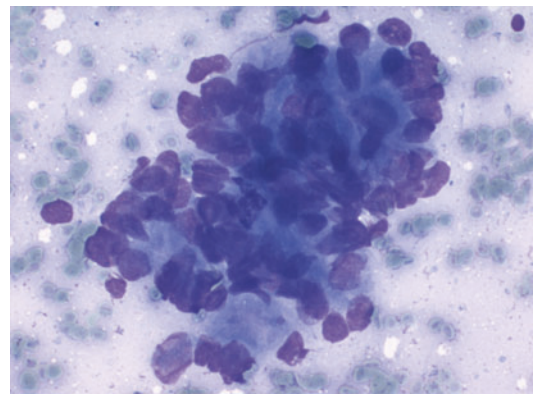
Benign hyperplasia, granulomatous prostatitis, overgrowth from poorly differentiated intestinal carcinomas (Figs. 1–4).



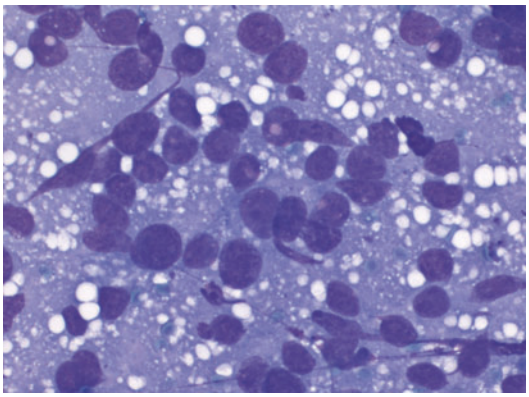
Prostate Adenocarcinoma, Cytological Findings, Fig. 1 Prostate cancer well differentiated: small tumor cells with round nuclei and distinct nucleoli. Acinar structures are always present. MGG



Prostate Adenocarcinoma, Cytological Findings, Fig. 2 Prostate cancer moderately well differentiated: tumor cells with oval to round nuclei in poorly formed acinar structures with cell overlapping. MGG



Prostate Adenocarcinoma, Cytological Findings, Fig. 3 Prostate cancer poorly differentiated: markedly enlarged tumor cells with fragile, irregular nuclei in dense clusters. MGG



Prostate Adenocarcinoma, Cytological Findings, Fig. 4 Prostate cancer undifferentiated: polymorphic round nuclei with distinct nucleoli in a *gray-blue* background of cytoplasmic fragments. MGG

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Prostatitis, Cytological Findings

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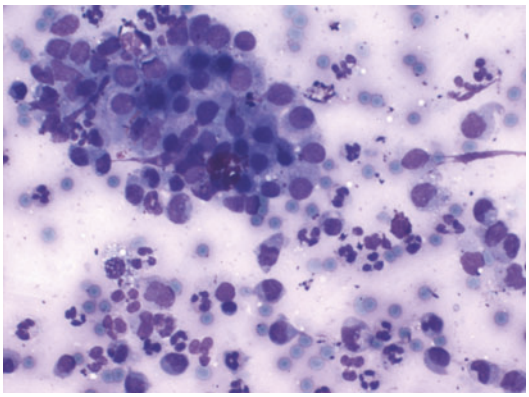
Synonyms

Acute-chronic and granulomatous prostatitis



Prostatitis, Cytological Findings, Fig. 1 Acute prostatitis: granulocytes, macrophages, cell debris, and monomorphic benign epithelial cells. MGG

Definition

Inflammatory cells with reactive and degenerative changes in the epithelial cells.

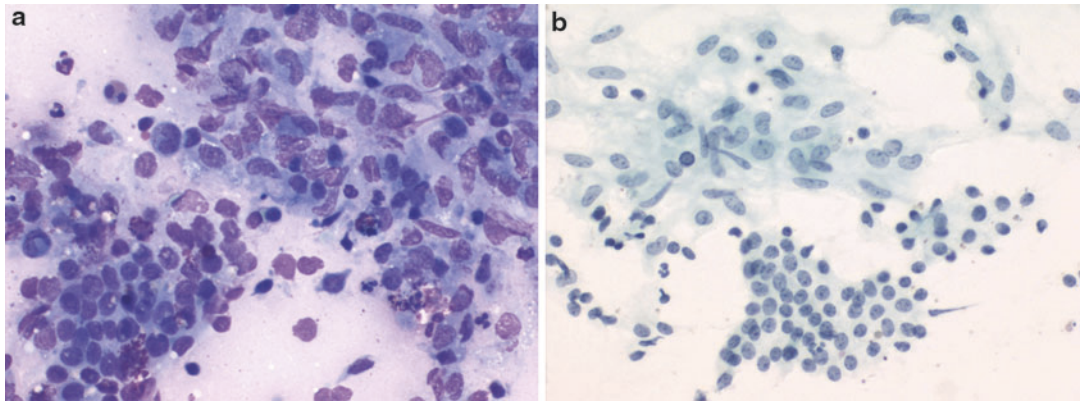
Clinical Features

- **Incidence**
  No exact figures are known but a prevalence of 2–10% has been reported.
- **Age**
  Middle aged to elderly.
- **Sex**
  It is an exclusive male disease.
- **Treatment**
  Acute bacterial prostatitis: antimicrobial treatment.

  Chronic bacterial prostatitis: 4–8 weeks of antimicrobial treatment.

  Chronic nonbacterial prostatitis: various types of treatment have been used with varying results.
- **Outcome**
  Acute bacterial prostatitis and chronic bacterial prostatitis: respond to adequate antimicrobials but recurrences are seen.

  Chronic nonbacterial prostatitis: questionable effect of therapy.



Prostatitis, Cytological Findings, Fig. 2 Granulomatous prostatitis: inflammatory cells of mixed-type epithelioid cells and benign epithelial cells. (**a**) MGG (**b**) Papanicolaou

Microscopy

Acute bacterial prostatitis: thin to viscous fluid is aspirated containing epithelial cells with degenerative changes, granulocytes, and macrophages.

Chronic bacterial prostatitis: sparse, bloody fluid is aspirated containing some lymphoid cells and degenerated epithelial cells.

Granulomatous prostatitis: acute and chronic inflammatory cells are found together with macrophages and epithelioid cells as well as few multinucleated giant cells. The epithelial cells may be degenerated but reactive changes mimicking carcinoma can be detected.

Differential Diagnosis

Benign hyperplasia (Figs. 1 and 2).

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Reactive Cellular Changes, Infections

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Synonyms

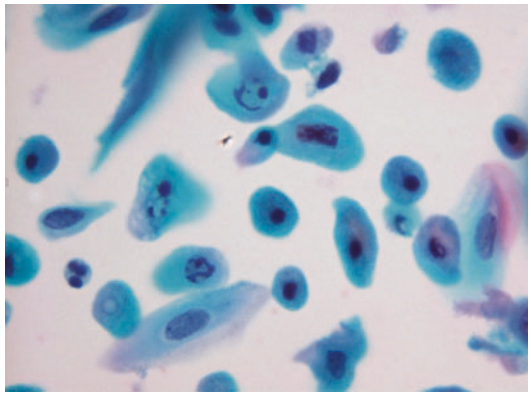
Inflammatory changes

Description

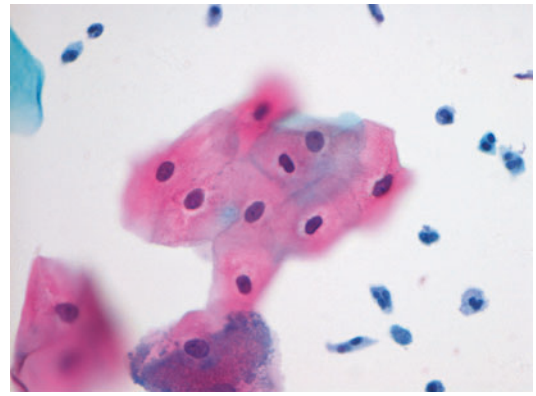
The morphological changes seen in cervical epithelial cells following cellular trauma can be quite variable, ranging from signs of cell death and degeneration on the one hand, to regeneration and repair on the other. Collectively, these changes are referred to as inflammatory changes. The term *reactive cellular changes* denotes any benign cellular alteration resulting from inflammation, which may be caused by infection, atrophy, radiation, intrauterine contraceptive device, or other nonspecific causes. Reactive cellular changes are therefore the morphological response of cells and tissues to injury. In the Bethesda System, reactive cellular changes can be included as a comment under the category "negative for intraepithelial lesion" (Solomon et al. 2002).

- Cytomorphology
  The cytomorphological changes of inflammation can be broadly divided into two groups: those associated with degeneration and cell death and those related to tissue regeneration and repair. Changes will be noted in both the nucleus and cytoplasm of the affected cells. Cell degeneration is typified by the breakup and eventual dissolution of the cell nuclei. Terms such karyorrhexis (nuclear fragmentation), karyopyknosis (nuclear shrinkage), and karyolysis (nuclear lysis) are commonly used to describe these alterations (Fig. 1). Other indicators of cell damage may include nuclear enlargement, vacuolation, margination (condensation of chromatin beneath the nuclear membrane), and wrinkling of the nuclear membrane. Cytoplasmic degeneration is commonly manifested as vacuolation, leucophagocytosis (ingestion of leucocytes), cytolysis, perinuclear haloing, and eosinophilia (Figs. 2–4). When injurious agents persist, cervical squamous epithelium may become thickened due to the accumulation of keratin. The terms hyperkeratosis, parakeratosis, and individual cell keratinization are used to describe the various forms of keratin accumulation in cells. In hyperkeratosis, the squamous epithelium develops a thick outer layer of keratin which may be apparent macroscopically as a whitish plaque

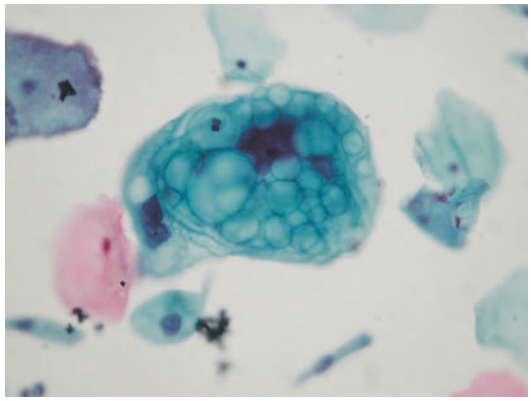
F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4



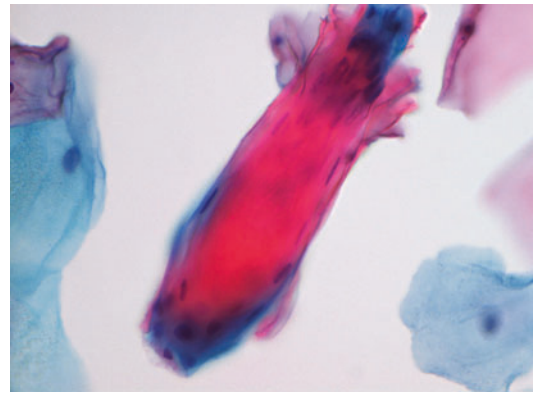
Reactive Cellular Changes, Infections, Fig. 1 Karyorrhexis, karyopyknosis, and karyolysis (Pap stain)



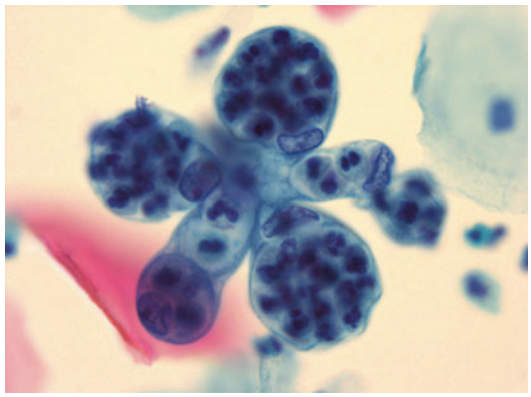
Reactive Cellular Changes, Infections, Fig. 4 Perinuclear haloes (Pap stain)



Reactive Cellular Changes, Infections, Fig. 2 Cytoplasmic vacuolation (Pap stain)



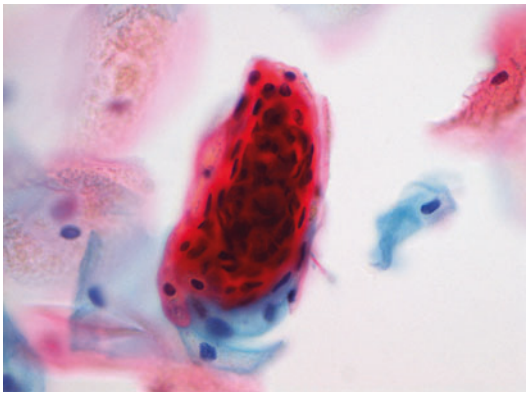
Reactive Cellular Changes, Infections, Fig. 5 Hyperkeratosis (Pap stain)



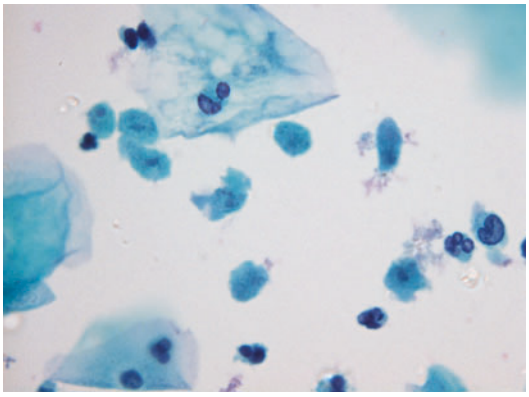
Reactive Cellular Changes, Infections, Fig. 3 Leucophagocytosis (Pap stain)

(leukoplakia) on the cervix. Cytologically, this is seen as sheets of anucleate squamous cells staining orange or yellow with the Papanicolaou method (Fig. 5). A characteristic feature of hyperkeratosis is the absence of nuclei in the keratin, distinguishing it from parakeratosis in which the nuclei persist in pyknotic form (Fig. 6).

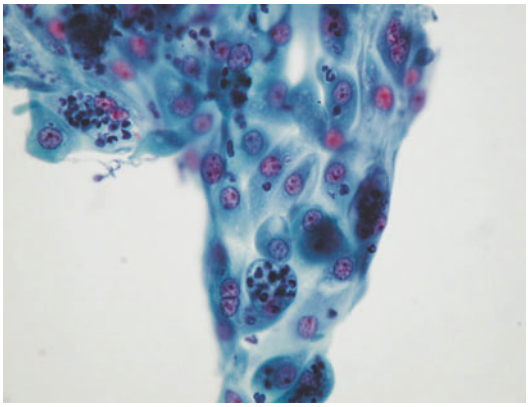
Cell repair and regeneration is often apparent as nuclear enlargement with hyperchromatic granular chromatin and prominence of nucleoli (Fig. 7). Cytoplasmic changes include vacuolation and the presence of cohesive sheets of cells with visible cell borders ("repair cells").



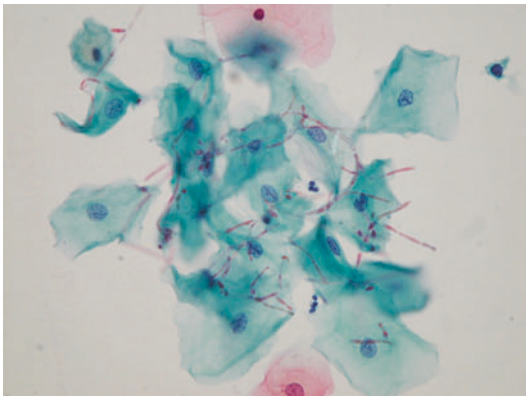
Reactive Cellular Changes, Infections, Fig. 6 Parakeratosis (Pap stain)



Reactive Cellular Changes, Infections, Fig. 8 *Trichomonas vaginalis* (Pap stain)



Reactive Cellular Changes, Infections, Fig. 7 Repair changes. Note visible nucleoli and granular but even chromatin (Pap stain)



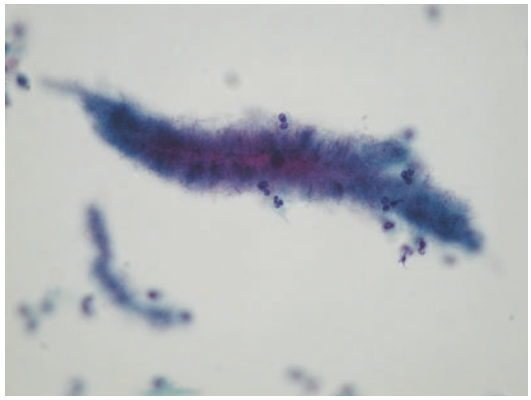
Reactive Cellular Changes, Infections, Fig. 9 *Candida* spp. (Pap stain)

- Infections
  Of all the microorganisms that may cause inflammation and infection in the cervix, only a small number are identifiable by cervical cytology. These include *Trichomonas vaginalis, Candida* spp., *Actinomyces*-like organisms, Bacterial vaginosis, Herpes simplex virus, and Human papillomavirus.

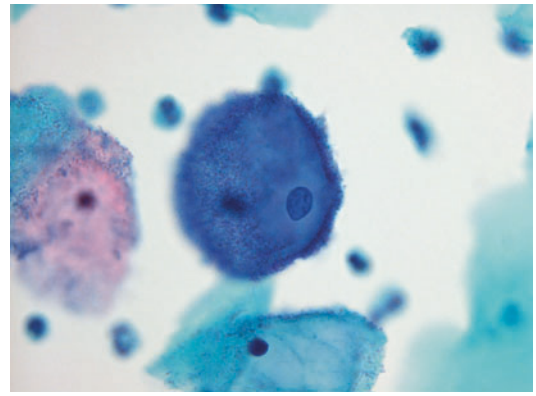
  Trichomonas vaginalis is a sexually transmitted protozoan causing vaginal itching and discharge. Trichomoniasis has an estimated prevalence rate of 3% in women of reproductive age (Sutton et al. 2007). However, as infection may be asymptomatic, the true prevalence is unknown. The organism possesses hair-like flagella which give it motility, and on high magnification, a smudged gray nucleus can be visualized (Fig. 8). Tiny pink granules may also be visible within the cytoplasm of the organism.

  Candida spp. is the only common fungus found in the lower genital tract. Approximately 75% of adult women will have vaginal candidiasis during their lifetime. The decision whether to treat a woman in whom *Candida* has been identified cytologically is a clinical one; in many instances, there are no symptoms and treatment is not required. The organisms are seen either as spores or as hyphae (Fig. 9).

  Actinomyces-like organisms are bacteria known to colonize intrauterine contraceptive devices without causing infection or specific symptoms. An estimated 10% of women with an intrauterine contraceptive device have



Reactive Cellular Changes, Infections, Fig. 10 *Actinomyces*-like organisms (Pap stain)

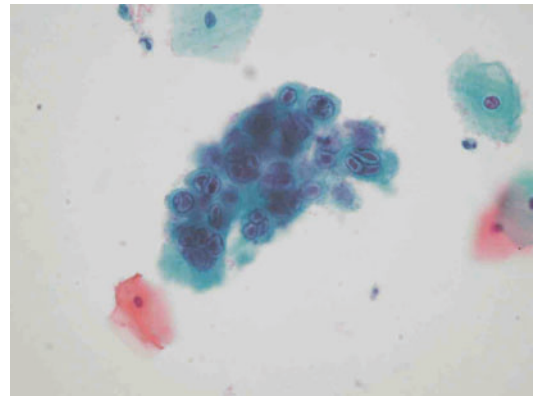


Reactive Cellular Changes, Infections, Fig. 11 A "clue cell" (Pap stain)

Actinomyces colonization (Chatwani and Amin-Hanjani 1994). The organisms can occasionally cause ascending infection and pelvic inflammatory disease. Actinomyces-like organisms are characterized by tangled masses of hematoxyphilic filaments in the Papanicolaou stain (Fig. 10).

Bacterial vaginosis is an inflammatory condition of the vagina caused by a mixture of anaerobic coccobacilli, including *Gardnerella vaginalis* and several other organisms. The organisms are considered to be a part of the normal bacterial population of the lower female genital tract, but they can sometimes proliferate and cause vaginal discharge. These bacteria have a tendency to cover the surface of squamous epithelial cells, giving them a hazy blue appearance and the name "clue cells" (Fig. 11).

Herpes simplex virus (HSV) causes highly infectious lesions of the mucosal surfaces, with or without symptoms. There are two types of HSV: Type 1 causes ulcerating lesions of the mouth, lips, eyes, and skin, whereas type 2 has a predilection for the genital and anal regions. An estimated 25% of women in the United States are seropositive for HSV type 2 infection (Fleming et al. 1997). HSV infection can be diagnosed cytologically with high specificity. The infection presents as multinucleated giant cells containing moulded nuclei with a pale, smooth chromatin texture ("ground glass" chromatin). Intranuclear viral inclusions may be visible as rounded eosinophilic structures in the center of the nucleus (Fig. 12).



Reactive Cellular Changes, Infections, Fig. 12 Herpes simplex virus effect (Pap stain)

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Reactive Cellular Changes, Intrauterine Contraceptive Device

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Synonyms

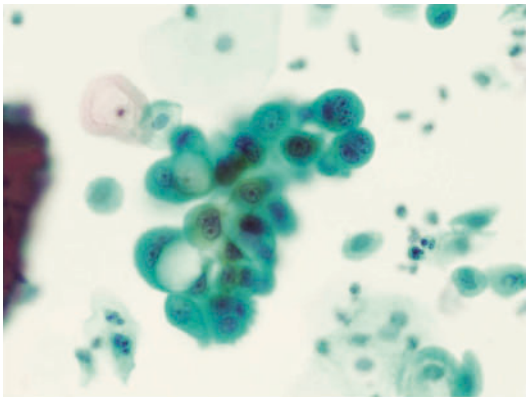
Intrauterine contraceptive devices; Intrauterine devices; IUCDs; IUD

Definition

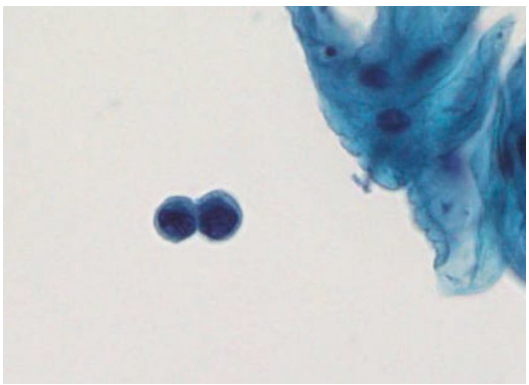
Intrauterine devices (IUCDs) are used as a form of contraception. Some release progesterone and are used as a treatment for primary menorrhagia in addition to contraception. Most of these are made from plastic or copper. This causes trauma to the lower uterine tract, which may lead to inappropriate shedding of endometrial cells, dysfunctional uterine bleeding, endometriosis, and pelvic inflammatory disease (Titmuss and Adams 2007). IUCD is also prone to colonization by Actinomyces (Titmuss and Adams 2007).

Microscopy

The smears may contain numerous polymorphs, histiocytes with bean-shaped nuclei, and clusters of rounded cells (squamous metaplastic and/or glandular endometrial cells), with cells showing large vacuoles that push the bland nucleus toward the edge of the cluster ("bubble-gum vacuoles") (Fig. 1) (Titmuss and Adams 2007). A few, small, scattered isolated cells with dark, but smudged, nuclei and smooth nuclear margins called ding cells can be seen (Fig. 2). Cellular enlargement, with cytoplasmic vacuoles, and nuclear



Reactive Cellular Changes, Intrauterine Contraceptive Device, Fig. 1 Endometrial cell clusters showing large vacuoles that push the bland nuclei toward the edge of the cluster ("bubble-gum vacuoles") (Pap ×100X)



Reactive Cellular Changes, Intrauterine Contraceptive Device, Fig. 2 Small cells with dark nuclei with smudged chromatin and smooth nuclear margins called ding cells (Pap ×100X)

enlargement, with prominent nucleoli, may be seen (Clayton 2008). The reactive changes may also include nuclear hyperchromasia with nuclear membrane irregularity and increased nuclear: cytoplasmic ratio (Clayton 2008). This may lead to overcalling cervical cytology samples as showing atypical squamous cells of undetermined significance (ASCUS) or atypical glandular cells of undetermined significance (AGUS).

Actinomyces are branching filamentous bacteria that are almost always associated with the presence of chronic endometritis, usually caused by an IUCD (Cobb 2008). Approximately 25% of patients with IUCDs may have Actinomyces present in their Pap specimens (Cobb 2008), Actinomyces may be associated with nonspecific inflammatory changes in the epithelial cells (Cobb 2008).

Differential Diagnosis

IUCD's effect on endometrial cells may be difficult to distinguish from atypical endometrial cells shedding from a well-differentiated adenocarcinoma.

Ding cells seen due to IUCD changes may be difficult to differentiate from small cell severe dyskaryosis.

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Reactive Cellular Changes, Repair

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Synonyms

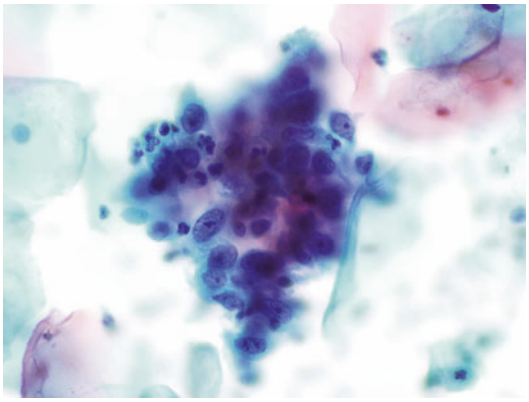
Regeneration; Repair

Reactive cellular changes due to regeneration and repair are associated with severe cervicitis, chronic infections, or surgical intervention. In most cases, atypical cells are found within the first 4 weeks following cryosurgery or any other surgical intervention, and the smears return to normal within 8 weeks (Hasegawa et al. 1975). The healing process may lead to changes in the squamous cells which may be difficult to distinguish from neoplasia (Bukovsky and Zidovsky 1985).

Cytomorphology

Most commonly, reactive cellular changes are seen in metaplastic squamous epithelial cells (Fig. 1) (Titmuss and Adams 2007). Reactive cellular changes are associated with (Titmuss and Adams 2007):

- Flat monolayered sheets of cells with maintained polarity
- Normal nuclear to cytoplasmic ratio



Reactive Cellular Changes, Repair, Fig. 1 A sheet of metaplastic cells showing nuclear enlargement with smooth thick nuclear membranes, evenly distributed chromatin, and prominent nucleoli along with the presence of polymorphs (PAP ×100X)

- Nuclei that are enlarged and round to oval in shape with smooth thick nuclear membranes
- Chromatin pattern is slightly coarse but evenly distributed
- Prominent nucleoli
- Cytoplasmic vacuolation

In majority of the cases, inflammatory cells in the form of polymorphs are present in the smears (Titmuss and Adams 2007), and in some cases, the cause of inflammation may be identified in the smears such as candida and *Trichomonas vaginalis*.

Differential Diagnosis

- Squamous dyskaryosis/glandular abnormality
- Invasive carcinoma because of large prominent nucleoli (Titmuss and Adams 2007).

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Reactive Lymphoid Hyperplasia, Cytological Findings

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Synonyms

Nonspecific lymphadenitis; Reactive lymphadenopathy; Specific lymphadenitis

Definition

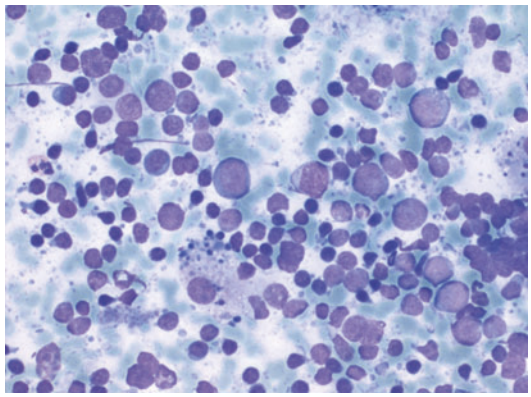
Follicular hyperplasia: lymph node with increased and hypertrophic germinal centers.

Interfollicular hyperplasia: proliferation and expansion of interfollicular areas.

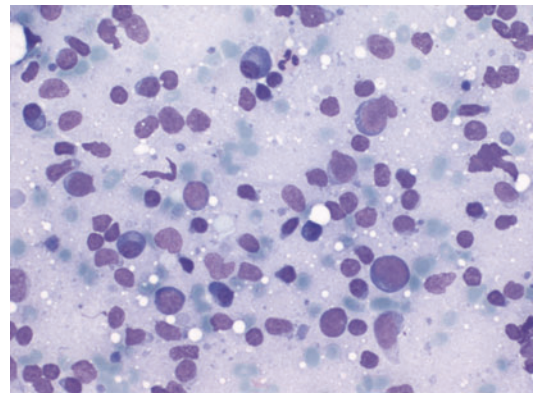
Marginal zone hyperplasia: proliferation of marginal zone lymphocytes in paracortical and interfollicular areas.

Clinical Features

- **Incidence**
  Relatively common.
- **Age**
  Children and young adults dominate. Rare in older patients.
- **Sex**
  No sex predilection.
- **Site**
  All sites but cervical nodes dominate clinically.



Reactive Lymphoid Hyperplasia, Cytological Findings, Fig. 1 Reactive lymphoid follicular hyperplasia: small mature lymphocytes, medium sized, and large centroblastic cells. One macrophage with tingible bodies (*center*). MGG



Reactive Lymphoid Hyperplasia, Cytological Findings, Fig. 2 Reactive interfollicular hyperplasia: small mature lymphocytes, immunoblasts, and plasma cells. MGG

- **Treatment**
  In case of bacterial infections, antibiotics may be necessary. Lymphadenitis caused by vaccination or viruses have no specific treatment.
- **Outcome**
  Self-limiting.

Microscopy

Follicular hyperplasia: polymorphic population of follicle center cells and tangible body macrophages (Fig. 1).

Interfollicular hyperplasia: granulocytes, small reactive lymphocytes, macrophages, immunoblasts, and plasma cells (Fig. 2).

Marginal zone hyperplasia: small to intermediate sized lymphocytes with rounded nuclei and rich pale cytoplasm ("monocytoid cells") and granulocytes.

Immunophenotype

Polyclonal B-cells, T-helper, and T-suppressor cells.

Molecular Features

No restriction of immunoglobulin or T-cell receptor genes.

Differential Diagnosis

Follicular hyperplasia: follicular lymphoma, marginal zone lymphoma, mantle cell lymphoma.

Interfollicular hyperplasia: Hodgkin lymphoma, nodal marginal zone lymphoma, Langerhans cell histiocytosis.

Marginal zone hyperplasia: marginal zone lymphoma.

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Renal Cell Carcinoma, Cytological Findings

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Synonyms

Hypernephroma

Definition

Renal cell carcinoma (RCC) originates in the lining of the renal tubules. Under this designation are included different subtypes with different morphologic features and occurring in different genetic settings.

Clear cell RCC is the most common type of kidney cancer in adult and it is frequently associated to a loss of genetic material in chromossome 3p, Von Hippel-Lindau (VHL) gene mutation. Despite the fact that a great majority of patients with VHL syndrome develop clear cell RCC, (33–50%), only a minority of clear cell RCCs occur in a VHL syndrome setting. Clear cell RCC is characterized by nests of malignant epithelial cells with clear or granular cytoplasm separated by a delicate capillary vasculature. In children clear cell RCC is uncommon as well as its association to VHL gene mutation. In childhood RCCs are characterized by various translocations involving the transcription factor E3 (*TFE3*) gene in chromosome Xp11.2 or they are inserted in a syndromatic clinical settings, as tuberous sclerosis, among others. In children, RCC has also been described in patients treated of neuroblastoma.

Clinical Features

Nowadays most cases of RCC are diagnosed before the classic triad of symptoms (hematuria, flank pain and abdominal mass) is present. Symptoms are indicative of advanced disease and bad prognosis. Cigarette smoking, hypertension and obesity are the strongest known risk factors.

- **Incidence**
  The incidence of RCC varies according to geographic location. North of Europe has higher incidence than North America and even higher when compared to Asia or Africa. Incidence has recently risen, due to incidental detection of small lesions, in abdominal imaging (Pascual and Borque 2008). Renal carcinoma in children accounts for 2–5% of all renal tumours and in about one third of the cases are associated to syndromes asVon Hippel Lindau and Tuberous Sclerosis (TSC). In 20% of the cases there is an association with specific translocations of chromosome Xp11.2.
- **Age**
  RCC affects children and adolescents most commonly beyond 10 year-old. In adult population, the average age at diagnosis is 60–64 years. Some tumours that were previously diagnosed as clear cell RCC in younger patients may in fact represent the Xp11 translocation RCC type.
- **Sex**
  RCC affects more the male population with a male-to-female ratio of 1.6:1.
- **Site**
  The site of origin of RCC is variable depending on its subtype. Clear cell RCC seems to arise from epithelial cells of the proximal convoluted tubules, within the renal cortex.
- **Treatment**
  Treatment is primarily surgical, with partial nephrectomy usually considered for tumours below 4 cm in diameter (stage pT1a) and radical nephrectomy for tumours larger than 4 cm. In metastatic disease cytokine immunotherapy with interleukin-2 is tried. More recently molecular targeting therapeutics seems more

promising. Antiangiogenic agents (sunitinib, sorafenib) and mTOR kinase inhibitors (temsirolimus) have been approved for treatment of renal cell carcinoma (RCC) in the United States and Europe.

- **Outcome**
  Patients with clear cell RCC tend to have a worse prognosis than patients with other histological subtypes of RCC. 5-year disease-specific survival rates are of 50–69%, compared with 67–87% for papillary RCC and 78–87% for chromophobe RCC. Patients with metastatic clear cell RCC have a poor prognosis with a 5-year disease-specific survival rate of 10.5%.

  Patients with RCC Xp11.2 associated are generally in an advanced stage of the disease at presentation and have poorer outcomes than TFE3 negative RCC (Rao et al. 2010).

Macroscopy

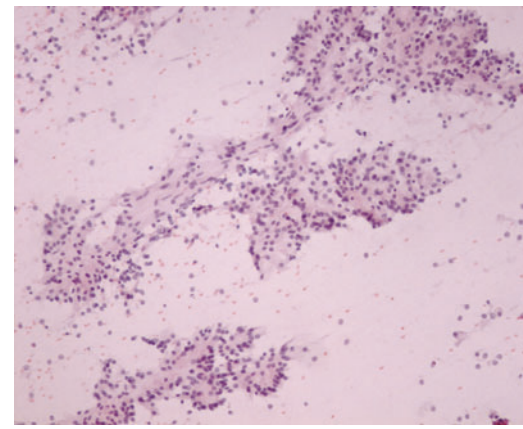
RCC clear cell type is a solitary tumour, well circumscribed and centred on the renal cortex. Tumour size ranges from 0.3 to 30 cm, with a mean of 6–7 cm. The tumour can have a capsule or pseudocapsule, and has pushing margins. Haemorrhage, necrosis, calcification and cystic changes are frequent. Extension into renal vein or vena cava is a common feature. The cutting surface of preserved areas has a typically golden colour because of the accumulation of cytoplasmic lipids.

Microscopy

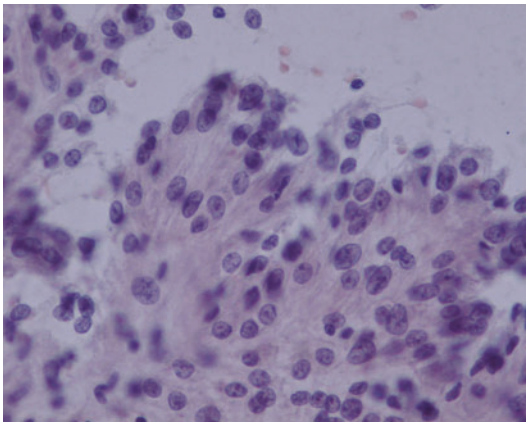
Histologically the most common type of RCC is clear cell type. These tumours are composed of different proportions of neoplastic cells with clear or granular cytoplasm. In histology neoplastic cells are disposed in alveolar or nested pattern. However, coexistence of more than one architectural pattern is common and focal regions of papillary or pseudo papillary architecture may also occur. Separating solid areas and nests there is a spreading fine capillary network. Nuclear atypia varies and is classified according to Fuhrman grade.

In paediatric age RCC are associated in 20% of the cases with specific translocations of chromosome Xp11.2. These RCC have a different architecture and show frequently a papillary or alveolar pattern. At least six different Xp11.2 translocation RCCs have been identified till now, all affecting the transcription factor E3 (*TFE3*) gene in chromosome Xp11.2. Depending on the type of translocation, there are minor morphologic details that can vary. In RCC with t(X;17), one of the more frequent translocations present in this subtype of RCC, architecture is in most cases alveolar or papillary; neoplastic cells are large and the cytoplasm can vary from clear to granular. Psammoma bodies as well as cytoplasmic hyaline inclusions are sometimes focally present.

Cytologic smears of clear cell type RCC are usually flooded with blood, and before you can obtain a representative sample, numerous passes must be done with fine needle biopsy. Large round and clear nude nuclei with prominent single nucleoli are in most situations the most frequent element (Figs. 1 and 2). In good representative samples, sheets of large polygonal neoplastic cells



Renal Cell Carcinoma, Cytological Findings, Fig. 1 Clear cell renal cell carcinoma. Material obtained by scraping the surface of the tumour. Numerous nude nuclei are present in the background. This figure also shows the presence of branching capillaries deeply associated to large polyedric neoplastic cells (H&E 100x)



Renal Cell Carcinoma, Cytological Findings, Fig. 2 Clear cell renal cell carcinoma. In this higher amplification nuclei detail is seen. Nuclei are round with prominent nucleoli (H&E 400x)

with well defined cytoplasmic borders are present. Cytoplasm can be whether clear whether eosinophilic and granular. All depends on the proportion of these two cell types. Cytoplasm has minute drops of glycogen and lipids, usually appreciated in Giemsa stains. The cytoplasm is fragile, which explains the quantity of nude nuclei in the smears, as well as a tigroid background aspect (Giemsa stains) (Fig. 1). This detail is shared with other tumours also containing glycogen in their cells cytoplasm, like seminoma, rhabdomyosarcoma and Primitive Neuroectodermal Embriogenic Tumor (Ewing/PNET) tumours (also called Ewing family tumours-EFTs). Sarcomatoid differentiation is seen in 5–25% of clear cell RCC and should be suspected whenever atypical fusiform cells are identified in necrotic smears.

In cytology, RCC Xp11.2 associated present, a characteristic morphology that reproduces histology. In most cases cells are Fuhrman II/III and as so neoplastic cells have large round nuclei with prominent eosinophilic nucleoli. Papillary architecture is seen as well as the presence of macrophages in the background. Psammoma bodies and calcifications as well as intracytoplasmic eosinophilic inclusions can also be appreciated in cytology.

Immunophenotype

Clear cell RCC tends to express the same immunoprofile that characterizes the cell of its provenance- the proximal convoluted tubule epithelium. As so it expresses low molecular weight cytokeratins (CK) characteristic of simple epithelia (8, 18), **C**luster **D**ifferentiation-CD10 and RCC marker. It also stains to **C**ytokeratins CK19 and CK7 in approximately 20% of the cases.

Expression of CK7 was observed in all of the recently reported clear cell RCC variants with smooth muscle stroma; most (87–100%) clear cell RCC strongly express vimentin.

Over expression of the epithelial marker EMA/MUC1 (Mucin 1, cell surface associated) is seen in 77–100% of clear cell RCC and the proportion of positive cells increases with tumor grade. Expression of E-cadherin, CD117/KIT, and parvoalbumin is less frequent or even absent. Summing up, the typical immunoprofile for clear cell type RCC is: vimentin+ /EMA+ /CD10+ /RCC marker+ /CK7- /CK19- /CD117- /E-cadherin- /parvoalbumin-. RCC Xp11.2 associated is characterized by immune nuclear labelling for TFE3 antibody and has a slightly different immunoprofile from classical clear cell RCC. CK7, EMA and vimentin are generally negative whether E-cadherin and CD10 are positive.

Molecular Features

Most clear cell RCCs are associated with genetic alterations involving chromosome 3p. Loss of genetic material in chromosome 3p occurs in 80–98% of sporadic clear cell RCC. Although alterations at 3p are believed to be the initiating genetic event, recent work suggests that clear cell RCC can subsequently progress along, at least, two distinct genetic pathways (Hoglund et al. 2004). The most common pathway (80% of clear cell renal cell carcinoma) mainly involves losses of entire chromosomes 3p together with gain of 5q via a translocation between chromosomes 3 and 5. The other pathway occurs in about 18% of clear cell RCC mainly involves gains of entire

chromosomes resulting in a hyperdiploid karyotype. Common gains involve chromosomes 7 (18–30%), 16 (11%), 20 (10%), 12 (10–15%), and 2 (9–14%).

The main inherited disorder predisposing for development of clear cell RCC is von Hippel-Lindau disease these patients have a germline mutation of the *VHL* gene at chromosome 3p25. In VHL disease renal carcinomas are frequently multifocal and/or bilateral and are always of the clear cell type.

Renal carcinoma in children account for 2–5% of all renal tumours and contrary to what happens in adult population, association to syndromes as Von Hippel Lindau occurs only in about one third of the cases.

In 20% of the cases, association with specific translocations of chromosome Xp11.2 (a member of the MiT transcription factor family) are found.

In a small proportion of cases, renal cell carcinoma in children has also been associated to neuroblastomas, nephroblastomas and WT1 gene, suggesting involvement of the Wnt signalling pathway in these last ones (Bruder et al. 2007).

Clear cell renal RCC may also arise in patients with tuberous sclerosis complex or Birt-Hogg-Dubè syndrome.

Differential Diagnosis

In cytology clear cell RCC raises differential diagnostic problems mainly in its variants. The predominance of granular eosinophilic cells should raise suspicion of oncocytoma, chromophobe RCC and papillary RCC type II.

In chromophobe RCC neoplastic cells bare a vegetable aspect with well defined cytoplasmic membrane giving the impression of a honeycomb pattern. Nuclei can be round and regular or else, irregular, with a typical raisinoid aspect. The nuclei are surrounded by a clear chromatin area (koilocytic appearance); the cytoplasm is not clear but fluffy.

Oncocytomas display monotonous polyedric oncocytic cells. No nude nuclei are present nor necrosis or mitotic figures.

Papillary RCC smears have a bloody background with macrophages. Papillary architecture is usually present and discriminative.

Immunostains and molecular studies are sometimes essential in separating these entities.

AMACR (**A**lpha **M**ethyl**A**cyl **C**oenzyme A **R**acemase) and CK7 are considered most useful for distinguishing clear cell RCC from papillary RCC.

Vimentin, CK7, CD117, E-cadherin and parvalbumin are considered most useful for distinguishing clear cell RCC from chromophobe RCC and oncocytoma.

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Renal Oncocytoma, Cytological Findings

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Synonyms

Tumor made of oncocytes

Definition

Oncocytes are epithelial cells characterized by an excessive amount of mitochondria which confer to the cells an abundant acidophilic, granular cytoplasm – from Greek "onkousthai" that means to swell and "cyte" that means cell. Renal oncocytomas are benign renal tumors derived from the collecting duct cells and so, sharing with chromophobe RCC the same cell origin. This nature explains the morphologic, immunophenotypic, ultrastructural and molecular similarities that both neoplasms share. On ultra structure the cytoplasm is filled with mitochondria and is devoid of any fat or glycogen vacuoles contrary to what is observed in clear cell RCC.

Clinical Features

Most patients are asymptomatic and it is not uncommon that the tumor is found accidentally in the course of ultrasound or abdominal CT scan (Kuroda et al. 2003). A small percentage of patients can refer flank pain, palpable mass, and hematuria or weight loss (Kuroda et al. 2003). Image diagnosis can be suspected by (1) the typical spoke-wheel pattern of feeding vessels around the tumor, (2) the presence of a central scar, (3) and sharp demarcation of the tumor with the same density of the surrounding renal parenchyma (Kuroda et al. 2003).

- **Incidence**
  Renal oncocytoma represents approximately 3–7% of adult renal epithelial tumors.
- **Age**
  Renal oncocytoma can affect any age although it affects mainly older patients.
- **Sex**
  Male female ratio is of 2:1.
- **Site**
  Renal oncocytomas can be bilateral, multifocal, and occasionally involve the kidney in a diffuse pattern (Theodosopoulos et al. 2009). Concomitant oncocytomas, and chromophobe tumors can coexist in Birt-Hogg-Dubè syndrome or rarely in a sporadic context. Coexistence with renal carcinoma or angiomyolipoma in tuberous sclerosis is reported.
- **Treatment**
  Partial nephrectomy or enucleation is considered curative.
- **Outcome**
  Oncocytoma is benign tumor with good prognosis.

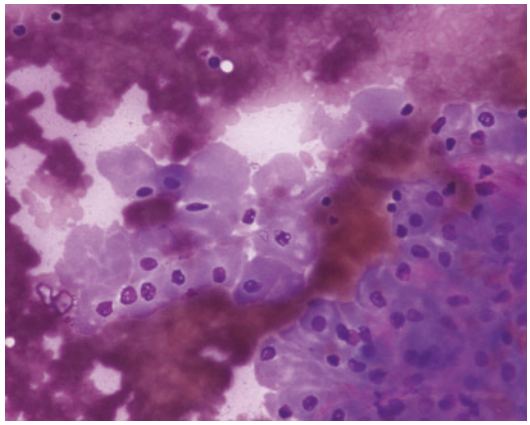
Macroscopy

The classical cut surface of an oncocytoma is homogeneous with no hemorrhage or necrosis and of a mahogany-brown color. In large tumors a central stellate fibrous scar is also characteristic of the cut surface. The tumor is well circumscribed and the size ranges from 0.6 to 15 cm.

Microscopy

On histology, the classical pattern is of nests, trabeculae, and tubulocystic groups laying in a myxomatous or hyalinized stroma. Cells, oncocytes, show abundant eosinophilic granular cytoplasm and nuclei are monotonous with small or occasionally large nucleoli (Fig. 1). Mitosis and necrosis are absent, whereas hemorrhage and cystic degeneration can be seen. Vascular invasion is rarely seen (Hes et al. 2008).

In cytological smears, neoplastic cells are large with abundant cytoplasm and are displayed single or in clusters; no stripped nuclei are seen. Nuclei are round with small or absence of nucleoli and occasionally atypia. Although the presence of large nucleoli is reported, this eventuality might not be considered a rule (Lazaro et al. 2006). Binucleation can also be present. The cytoplasm is eosinophilic and granular with well-defined borders, even if not as well defined as in chromophobe renal cell carcinoma.



Renal Oncocytoma, Cytological Findings, Fig. 1 Renal oncocytoma fine-needle aspiration. Neoplastic cells are large with abundant cytoplasm and are displayed single or in clusters. Nuclei are small and round with small or no nucleoli (Giemsa 400×)

Immunophenotype

Immunophenotype is shared with chromophobe renal cell carcinoma. Both tumors derived from the intercalated cell of the collecting duct system. Most oncocytomas are negative for Hale's colloidal iron. Oncocytomas are positive for **e**pithelial **m**embrane **a**ntigen – EMA, keratins 8 and18, (both oncocytoma and chromophobe RCC can express a dot-like pattern), keratin 14, and in a variable and focal way to keratin 7 and 20 vimentin, parvalbumin, **c**luster **d**ifferentiation-CD117, E-cadherin, and kidney-specific cadherin are positive. Oncocytoma is, in most cases, negative to N-cadherin and CD10.

Molecular Features

In chromosomal analysis, oncocytomas present a range of molecular alterations. The most frequent chromosome abnormality seen includes combined loss of chromosomes 1 and X/Y (Yusenko 2010). Another set of oncocytomas can show translocation involving chromosome 11, with a breakpoint at 11q12-13 (Yusenko 2010). Other more rare chromosome rearrangements have been reported, such as t(1;12)(p36;q13), loss of chromosome 14, and gain of chromosome 12 (Yusenko 2010).

Differential Diagnosis

Differential diagnosis should be done with chromophobe renal cell carcinoma and clear cell renal cell carcinoma (eosinophilic variants). This has already been discussed in the respective items.

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Reserve Cell Hyperplasia, Cytological Findings

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Synonyms

Physiological hyperplasia

Description

Physiology

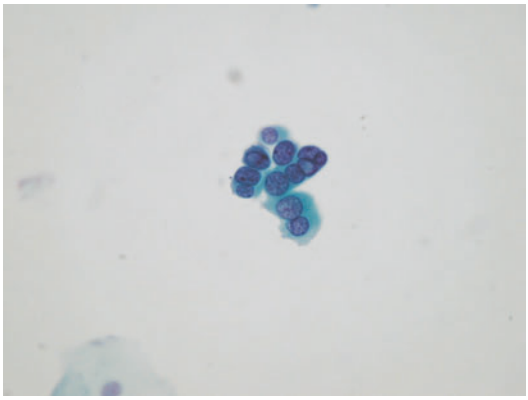
In the lower female genital tract, reserve cells are small undifferentiated cells found as a single layer beneath the endocervical columnar epithelium. They are thought to be derived from Mullerian epithelial cells and have the capacity to transform into both endocervical columnar and squamous epithelium in the endocervix (Martens et al. 2007). Reserve cell hyperplasia is an adaptive response of the endocervix to injury, whereby the reserve cells proliferate to replace the overlying epithelial cells. The process occurs because of normal physiological changes that influence the size and shape of the cervix throughout reproductive life. Before puberty, the cervix is relatively small and stable. At the onset of puberty, and periodically thereafter, the cervix undergoes dramatic changes in volume under the influence of ovarian hormones. An increase in cervical volume causes the endocervical columnar epithelium to evert, a process known as ectopy or ectropion. Exposure of the delicate endocervical epithelium to the acid environment of the vagina is the initiating stimulus for reserve cell hyperplasia. The hyperplastic epithelium subsequently differentiates into protective squamous epithelium via the process of squamous metaplasia. When the process is complete, the zone of exposed endocervical epithelium is replaced by squamous epithelium.

Histology

When quiescent, endocervical reserve cells can be seen as an inconspicuous single layer of small round cells sitting on the basement membrane in the endocervical canal. Reserve cell hyperplasia, however, is evident as multiple layers of undifferentiated cells completely obliterating the overlying endocervical epithelium. Occasionally, a single layer of remnant endocervical columnar cells can be seen on top of an area of reserve cell hyperplasia.

Cytology

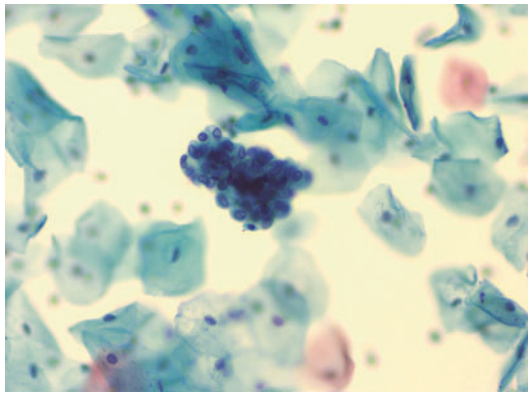
Cytologically, reserve cells are small round cells with a high nuclear-to-cytoplasmic ratio and smooth, even chromatin, often appearing as cell clusters (Fig. 1) but occasionally as single cells. Morphologically, they are similar in appearance to basal cells of squamous epithelium.



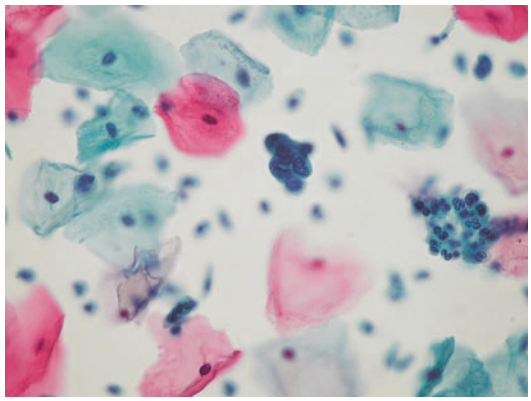
Reserve Cell Hyperplasia, Cytological Findings, Fig. 1 Reserve cells (Pap stain)

Differential Diagnosis

Although reserve cells are clinically insignificant and need not be reported, they may be a source of interpretative difficulty and can closely mimic neoplastic cells. Although the nuclei of reserve cells can be quite hyperchromatic, close attention to their uniform chromatin pattern and smooth nuclear membranes should prevent misinterpretation in the vast majority of cases. When present as densely packed cell clusters, reserve cells can also resemble endometrial cells (Fig. 2). An important distinction between the two cell types is the often papillary configuration of endometrial cells and their wrinkled nuclear membranes. Occasionally, reserve cells may detach from their parent cell clusters because of cytological processing. When this happens, they may be mistaken for lymphocytes. However, in cervical cytology practice, their distinction is rarely of any clinical significance. Of crucial clinical importance is the accurate distinction between reserve cells and high-grade squamous



Reserve Cell Hyperplasia, Cytological Findings, Fig. 2 Endometrial cells (Pap stain)



Reserve Cell Hyperplasia, Cytological Findings, Fig. 3 Cells from HSIL can bear a close resemblance to reserve cells (Pap stain)

intraepithelial lesion. Cells of the latter have coarsely granular chromatin and irregular shapes (Fig. 3), distinguishing them from reserve cells, which have even chromatin and smooth nuclear borders.

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Rhabdoid Tumor, Cytological Findings

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Synonyms

Atypical teratoid rhabdoid tumor, when diagnosed in the central nervous system; Rhabdomyosarcomatoid variant of wilms

Definition

MRT is one of the most aggressive and lethal malignancies in pediatric oncology high-risk tumors European Society of Paediatric Oncology (SIOPE-2001-revised working classification of renal tumors of childhood). These tumors have a controversial histogenesis and the cell of origin is yet to be established. Molecularly these tumors have deletions in chromosome 22 involving the INI1 tumor suppressor gene.

Clinical Features

In the kidney MRT is diagnosed, usually as an abdominal palpable mass. Seventy-five percent of the patients have microscopic hematuria, and approximately 25% of patients have proteinuria and hypercalcemia, due to tumor production of parathormone and parathormone-like substances.

Abdominal CT shows a large, hemorrhagic/necrotic lobulated mass in the center or periphery of the kidney. Calcification occurs frequently, these are often linear or curvilinear, and they may outline tumor lobules.

About 15% of the patients will have a primary tumor of the central nervous system resembling a Primitive Neuroectodermal Tumor (PNET) (Argani and Ladanyi 2003; Bonim et al. 1984). Generally these tumors are relatively small as they metastasize early (Argani and Ladanyi 2003).

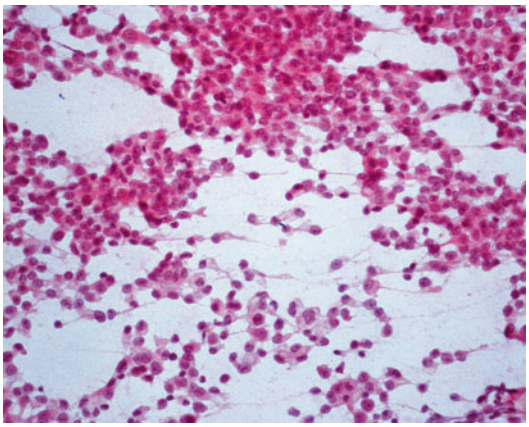
- **Incidence**
  These tumors account for less than 2% of pediatric renal tumors (Argani and Ladanyi 2003).
- **Age**
  Rhabdoid tumors have a predilection for infants and very young children. Eighty percent of the patients are younger than 2 years.
- **Sex**
  Males seem to be slightly more affected than females – sex ratio 1.5M/1F.
- **Site**
  Most tumors are described in the kidney. MRT can be practically appearing in every location in the body, including the brain, liver, kidney, soft tissues, lung, skin, and heart. In 15% of the cases, they are synchronous or metachronous to genetically independent primary embryonal brain tumors (PNET-like neoplasm) (Argani and Ladanyi 2003).
- **Treatment**
  Treatment approach has two distinguish pathways. In Europe these tumors are considered as high-risk tumors (SIOPE-2001-revised working classification of renal tumors of childhood) and so treated as protocol with primary chemotherapy followed by nephrectomy. The renal tumor committee of the children's oncology group (COG) advocates nephrectomy followed by chemotherapy. Radiotherapy is reserved in both protocols for stage III and IV patients.
- **Outcome**
  Metastases are frequent, mainly to the lung, and the prognosis is usually bad with 80% of deceases within the first 2 years. Some studies refer a better outcome for girls.

Macroscopy

These tumors are bulky, lobulated, friable, solid, gray-tan masses with areas of necrosis and hemorrhage.

Microscopy

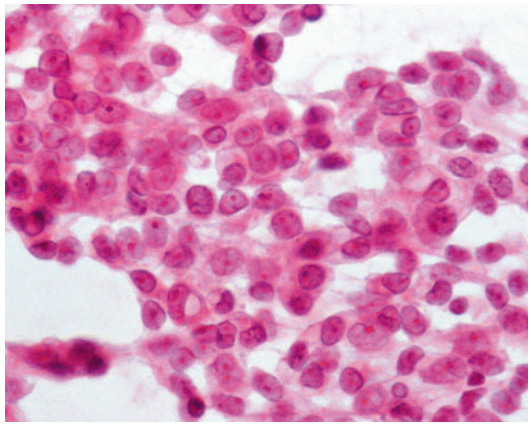
Malignant rhabdoid tumors are characterized by infiltrative sheets or solid trabeculae of large tumor cells with vesicular chromatin, nuclei with prominent nucleoli, moderate amounts of eccentric eosinophilic cytoplasm, and a distinctive, globoid, hyaline pink intracytoplasmic inclusions that correspond to paranuclear cytoplasmic whorls of intermediate microfilaments, seen on electronmicroscopy. Mitoses are frequent as well as necrosis. Vascular invasion is extensive. This classical pattern can alternate with small round blue cell foci, sclerosing (including chondroid), epithelioid, spindled, lymphomatoid, or histiocytoid patterned areas.



Rhabdoid Tumor, Cytological Findings, Fig. 1 Round to polygonal rhabdoid-like dispersed cells or in clusters. The cells have rhabdoid features with eccentric pale nuclei with reticular chromatin and prominent single eosinophilic macronucleoli (H&E 200×)

On cytology a necrotic background with numerous bare nuclei and rhabdoid cells can be seen. Rhabdoid cell nuclei are characteristically eccentric with clear and condensed chromatin and a single eosinophilic macronucleoli surrounded by a clear area (Fig. 1) (Akhatar et al. 2006; Barroca et al. 2003; Drut 1990; Shariifah 1994). The cytoplasm is fragile and whenever preserved is eccentric and pale or with paranuclear intracytoplasmic eosinophilic inclusions (Fig. 2) (Akhatar et al. 2006; Barroca et al. 2003; Drut 1990; Shariifah 1994).

The most useful ultrastructural finding is a large whorl of intermediate filaments in the cytoplasm* with a diameter of 8–10 nm, correlating with the rhabdoid inclusions seen with light microscopy.



Rhabdoid Tumor, Cytological Findings, Fig. 2 Neoplastic cells show a clear area around the macronucleoli. The cytoplasm is fragile and pale. Occasionally intracytoplasmic eosinophilic inclusions are seen (H&E 400×)

Immunophenotype

Attention must be paid to false-positive staining due to antibody trapping by the cytoplasmic whorled filaments. Most tumors are positive for vimentin and epithelial membrane antigen (EMA) and/or cytokeratin. Positivity for glial fibrillary acidic protein, neuron-specific enolase (NSE), smooth muscle actin, desmin, CD99, synaptophysin, and other markers has been seen in malignant rhabdoid tumor. CD56 is generally negative. Malignant rhabdoid tumor lacks INI1 immunohistochemical staining, whereas most other tumors have detectable INI1 protein.

Molecular Features

Most tumors are sporadic, but in some rare situations there is a familiar rhabdoid predisposition syndrome, associated with a germ-line mutation of INI1 gene. These children are more prone to develop malignant rhabdoid tumors, atypical teratoid tumors, choroid plexus carcinomas, ▶ medulloblastomas, and ▶ primitive neuroectodermal tumors. MRT are characterized by chromosome 22 deletion despite its anatomical location. This deletion leads to the inactivation of INI1 gene. INI1 gene seems to be involved in chromatin remodelling and so predispose to chromatin instability and polyploidization. INI1 also interacts with cyclin D1 and consequent G1 cell cycle arrest (Simons et al. 1999). This interaction seems to be involved on the initial steps of cancerization in rhabdoid tumors.

Differential Diagnosis

Cytological differential diagnosis of MRT can be very demanding as the rhabdoid phenotype is not exclusive to this entity and is shared by other pediatric tumors, namely, ▶ PNET, clear cell sarcoma of the kidney (CCSK), ▶ congenital mesoblastic nephroma, renal medullar carcinoma, ▶ rhabdomyosarcoma, and even ▶ neuroblastoma (Weecks et al. 1991). Except for renal medullar carcinoma (also negative for INI1 antibody), all the other referred entities are positive for this antibody. Likewise, extensive sampling of all the aforementioned entities will show typical areas that will able the accurate diagnosis. In difficult cases molecular studies can help excluding PNET and alveolar rhabdomyosarcoma.

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Rhabdomyosarcoma, Cytological Findings

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Synonyms

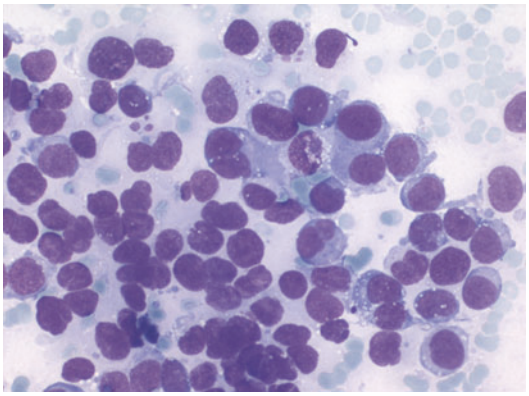
Alveolar rhabdomyosarcoma; Embryonal rhabdomyosarcoma; Pleomorphic rhabdomyosarcoma

Definition

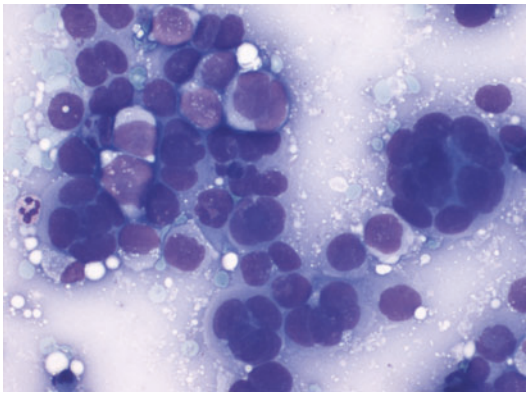
Alveolar rhabdomyosarcoma (ARS), primitive round cell tumor which shows partial skeletal muscle differentiation. Embryonal rhabdomyosarcoma (ERS), primitive sarcoma which shows features of embryonic muscle cells. Pleomorphic rhabdomyosarcoma (PRS), high-grade sarcoma which shows evidence of skeletal muscle differentiation (Figs. 1–3).

Clinical Features

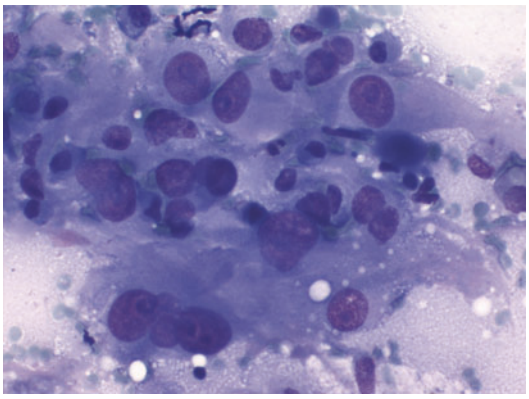
- **Incidence**
  ARS, 1/1000 000; ERS, 3/1000 000; PRS, 1/1000 000.
- **Age**
  ARS: all ages but teenagers and young adults dominate. ERS: children. PRS: median-aged patients.
- **Sex**
  ARS: no sex predilections. ERS: slight male predominance. PRS: male predominance.



Rhabdomyosarcoma, Cytological Findings, Fig. 2 Embryonal rhabdomyosarcoma: round tumor cells some with distinct blue cytoplasm with small vacuoles and eccentric nuclei. MGG



Rhabdomyosarcoma, Cytological Findings, Fig. 1 Alveolar rhabdomyosarcoma: round tumor cells with monomorphic nuclei often peripherally oriented. The cytoplasm is often rich and grey-blue with vacuoles. Cell clusters and alveolar structures can always be found. MGG



Rhabdomyosarcoma, Cytological Findings, Fig. 3 Pleomorphic rhabdomyosarcoma: large tumor cells with nuclei of varying size and shape. Typically, many cells have a rich grey-blue elongated cytoplasm. MGG

- **Site**
  ARS: extremities, paraspinal region, and paranasal sinuses. ERS: head and neck, genitourinary tract, and various visceral organs. PRS: lower extremities dominate.
- **Treatment**
  ARS: chemotherapy, surgery, and radiation. ERS: surgery, chemotherapy, and radiation. PRS: surgery and radiation.
- **Outcome**
  ARS, ERS: younger patients with low-stage disease have the best chance of survival. PRS: poor prognosis.

Microscopy

ARS: small, monomorphic cells with round to oval nuclei and sparse cytoplasm with few vacuoles. Mostly dispersed cells but alveolar structures are often present. Few cells with rhabdomyoblastic differentiation. ERS: spindle and round cells with sparse cytoplasm and irregular nuclei with distinct nucleoli. Cells with elongated eosinophilic grey-blue cytoplasm resembling rhabdomyoblasts. PRS: large pleomorphic cells with rich cytoplasm and nuclei with distinct nucleoli.

Immunophenotype

ARS, ERS: vimentin, desmin, and actin positive. Distinct nuclear staining for MyoD1. PRS: most cases express vimentin, desmin, and actin. MyoD1 may be positive.

Molecular Features

ARS: t(2;14) or t(1;13). ERS: various complex structural and numerical changes have been reported. PRS: no consistent pattern reported.

Differential Diagnosis

ARS: Ewing/PNET, precursor lymphoma, and poorly differentiated synovial sarcoma. ERS: leiomyosarcoma and alveolar rhabdomyosarcoma. PRS: MFH, pleomorphic liposarcoma, leiomyosarcoma, and high-grade peripheral nerve sheet tumors.

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Rheumatoid Arthritis (RA), Effusions Associated with

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Synonyms

Rheumatoid effusions

Definition

Pleural effusions are not related to the severity or duration of the disease and may precede

or occur simultaneously with the onset of the joint disease (Montes and Guarda 1987; Boddington et al. 1971).

Clinical Features

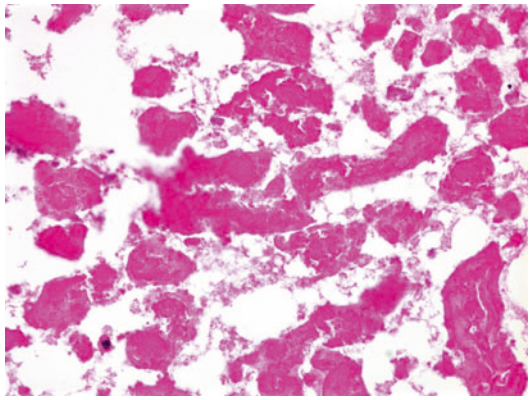
- **Incidence**
  Most patients with RA have small amount of effusions and are generally asyptomatic (Balbir-Gurman et al. 2006). Symptomatic pleural effusions occurring in association with RA have been described in 3–5% of RA patients although pleural involvement based on chest radiograms is much higher (Ferreiro et al. 2011).
- **Age**
  Almost all reported RA effusions occurred in patients over 35 years old.
- **Sex**
  Even though the disease has a female preponderance, effusions are more commonly seen in the male population constituting up to 80%. About 80% of those cases also had rheumatoid nodules.
- **Site**
  Effusions in rheumatoid arthritis are usually pleural and less commonly pericardial or peritoneal. The majority of patients present with unilateral effusion (>70%), most commonly on the left. However, bilateral or migratory effusion from one side to the other is not rare.
- **Treatment**
  The treatment of RA effusions is controversial. Repeated pleural injections with corticosteroids are usually performed, but its effectiveness has not been established. It is believed that high intra-pleural steroid injections may provide temporary control until systemic remission is achieved through suppressive therapies.
- **Outcome**
  The prognosis is variable. Small asymptomatic effusions usually resolve spontaneously. Large effusions usually have a more protracted course and take several months if they do resolve. Resolution occurs in up to 50% of these large effusions. In the remaining patients, the effusions may persist for years. Unresolved effusions may result in further complications such as marked pleural thickening, trapped lung, and even progressive restriction of the lung volume. Bacterial empyema may superimpose.

Macroscopy

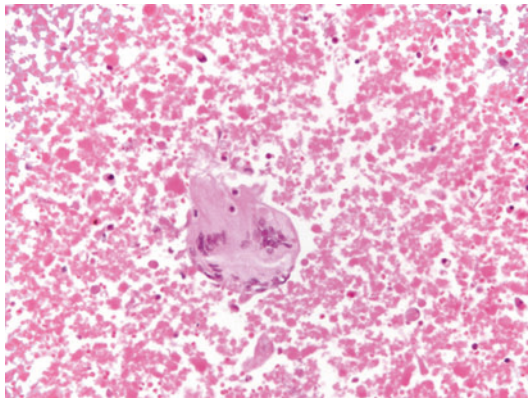
Pleural effusions are usually small to moderate in size, and the fluid is usually yellow-green but may be turbid, opaque, clear, straw-colored, and occasionally bloody (Katikireddy et al. 2005; Balbir-Gurman et al. 2006).

Microscopy

The fluid is usually exudative in nature and characterized by a high protein level (>4 g/dL), a very low glucose level (<40 mg/dL), pleural acidosis (PH < 7.2), high levels of lactate dehydrogenase (>700 IU/L), high cholesterol levels (>65 mg/dL), and negative bacterial smears or cultures (Katikireddy et al. 2005; Balbir-Gurman et al. 2006). Positive serological tests for rheumatoid factor in the pleural fluid, often to a higher titer than in the serum, have been used as a diagnostic aid (Boddington et al. 1971). Cytologically, the pleural fluid has a unique inflammatory picture that closely parallels the histological findings observed in rheumatoid nodules (Montes and Guarda 1987). The necrotic center of the rheumatoid nodule gives rise to the typical background of granular, amorphous, and necrotic debris which stains blue to pink to orange and has a fluffy appearance (Fig. 1). The palisading epithelioid histiocytes give rise to the large slender, spindle, elongated, or carrot-shaped cells that often have tadpole shapes. The spindle cells show various degrees of degeneration (Montes and Guarda 1987; Boddington et al. 1971). Multinucleated giant cells (Fig. 2) appear as either slender and elongated or rounded cells (Balbir-Gurman et al. 2006). The smears also contain scattered ghost anucleated cells, and other cellular elements such as neutrophils, mononuclear cells, and



Rheumatoid Arthritis (RA), Effusions Associated with, Fig. 1 High-power Papanicolaou stain demonstrates chunks of acellular granular, necrotic, amorphous debris (Pap stain)



Rheumatoid Arthritis (RA), Effusions Associated with, Fig. 2 High-power H&E stain of cell block demonstrates a multinucleated giant cell in a background of acellular granular debris (H&E stain)

macrophages (Montes and Guarda 1987; Boddington et al. 1971). The triad of slender or elongated cells, multinucleated giant cells, and necrotic background material together with a rarity or absence of mesothelial cells is diagnostic of rheumatoid pleurisy (Montes and Guarda 1987; Balbir-Gurman et al. 2006). Patients with rheumatoid arthritis can have nonrheumatoid pleural effusions. These patients usually have another cause for the pleural effusion such as pneumonia, congestive heart failure, or malignancy. The fluid of these patients does not have the cytologic triad described previously (Montes and Guarda 1987).

Molecular Features

High levels of pleural SC5b-9 and low levels of C3 and C4 in these fluids are indicative of local autoimmune activation of the inflammatory process in rheumatoid pleurisy.

Differential Diagnosis

The elongated tadpole cells, however, raise the differential diagnosis of squamous cell carcinoma and sarcomas metastatic to the pleura. Squamous cell carcinomas involve serous surfaces occasionally; they can shed single tadpole cells, some with glassy keratinized appearance, but also small tumor cell clusters. Sarcomas may present as elongated cells also, but these are often large, bizarre, and multinucleated with hyperchromatic nuclei and multiple large nucleoli (Montes and Guarda 1987).

Cross-References

- ▶ Systemic Disease and Effusions, Cytological Findings
- ▶ Systemic Lupus Erythematosus (SLE), Effusions Associated with

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Rosai-Dorfman Disease, Cytological Findings

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Synonyms

Sinus histiocytosis with massive lymphadenopathy

Definition

Rosai-Dorfman disease is a rare histiocytic disorder described in 1969 as sinus histiocytosis with massive lymphadenopathy by Rosai and Dorfman. The cause is unknown, but it has been speculated that it can be an infectious or autoimmune disease.

Clinical Features

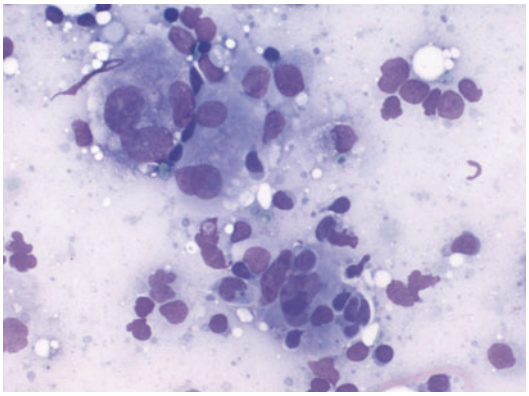
- **Incidence**
  Very rare disease.
- **Age**
  It occurs in any age group, but it is most frequent in children and young adults.
- **Sex**
  Males and individuals of African descent dominate.
- **Site**
  Lymph node of the neck is the most common localization. Usually, they are bilateral, often bulky, painless cervical lymphadenopathy, but axillary, mediastinal, and para-aortic nodes can be involved. Extranodal manifestations occur in skin, upper respiratory tract, and the sinuses.
- **Treatment**
  Since the disease is self-limited, most of patients should be followed closely without any active therapy. Some patients with systemic symptoms or those with sudden enlargement of nodes may be treated with prednisone. For patients with vital organ compression, surgery and high-dose corticosteroids should be tried first, but radiotherapy may be needed in resistant cases or whenever surgery is not feasible.
- **Outcome**
  Most cases regress spontaneously.

Microscopy

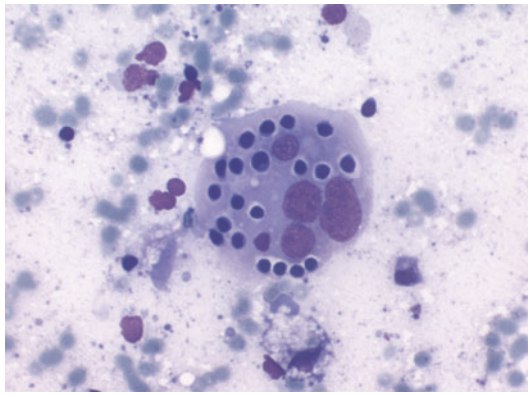
The FNA smears contain of plasma cells, lymphoid cells, and large histiocytes with abundant pale, eosinophilic cytoplasm containing well-preserved lymphocytes (lymphophagocytosis, emperipolesis) that is characteristic of this benign disorder (Figs. 1 and 2).

Immunophenotype

The histiocytes show reactivity for S-100 protein and alpha-1-antichymotrypsin but negative for lysozyme.



Rosai-Dorfman Disease, Cytological Findings, Fig. 1 Rosai-Dorfman disease: FNA cytology of a node presents large histiocytes with abundant cytoplasm and few mature lymphocytes. MGG



Rosai-Dorfman Disease, Cytological Findings, Fig. 2 Rosai-Dorfman: FNA of a node presents a multinucleated histiocyte with large cytoplasm containing mature lymphocytes (lymphophagocytosis/emperipolesis). MGG

Molecular Features

No chromosomal changes known.

Differential Diagnosis

Other histiocytic disorders (Langerhans cell histiocytosis, sinus histiocytosis, histiocytic sarcoma), lymphoma, metastatic melanoma, and carcinoma.

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Round Cell Sarcomas, Cytological Findings

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Variants

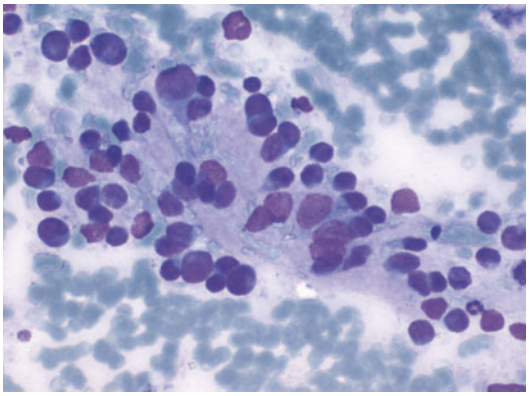
Neuroblastoma, Ewing sarcoma/primitive neuroectodermal tumor (PNET), Desmoplastic small-round-cell tumor, Alveolar rhabdomyosarcoma

Definition

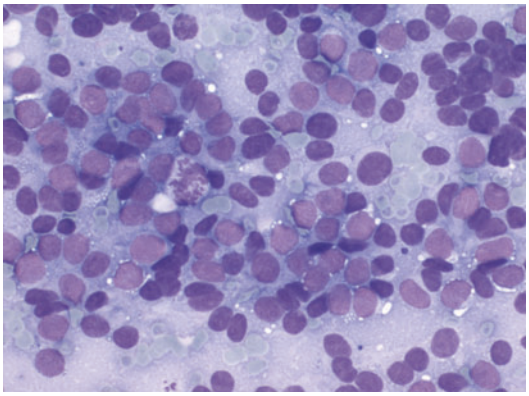
Primitive malignant round cell neoplasms with different and varying degrees of differentiation.

Clinical Features

- **Incidence**
  Neuroblastoma: 2–3/1,000,000
  Ewing sarcoma/PNET: 1/1,000,000
  Desmoplastic small-round-cell tumor: rare
  Alveolar rhabdomyosarcoma: 1/1,000,000.
- **Age**
  Childhood and young adults.
- **Sex**
  Slight predominance for male gender except for desmoplastic small-round-cell tumor which shows a striking male predominance.
- **Site**
  Neuroblastoma: adrenal medulla, neck mediastinum, retroperitoneum.
  Ewing sarcoma/PNET: diaphysis of long bones.
  Desmoplastic round cell tumor: abdominal cavity.
  Alveolar rhabdomyosarcoma: extremities, perineal region, paranasal sinuses.
- **Treatment**
  Chemotherapy, surgery, radiation.



Round Cell Sarcomas, Cytological Findings, Fig. 1 Neuroblastoma: round tumor cells with irregular nuclei and sparse cytoplasm. The cells are often loosely attached to a fibrillar network of neutrophil forming rosette-like structures. MGG



Round Cell Sarcomas, Cytological Findings, Fig. 2 Ewing sarcoma: tumor cells of two types – one with small hyperchromatic nuclei and with sparse or no cytoplasm. The second type is larger with round to oval nuclei and distinct cytoplasm with few clear vacuoles. A picture of mitosis is present. MGG

- **Outcome**
  Neuroblastoma: 80%survival in children younger than 1 year and 30% 5-year survival in children with high risk Stage 4.
  Ewing/PNET: survival rate around 40%.
  Desmoplastic small-round-cell tumor: poor survival.
  Alveolar rhabdomyosarcoma: survival rate of 70%.

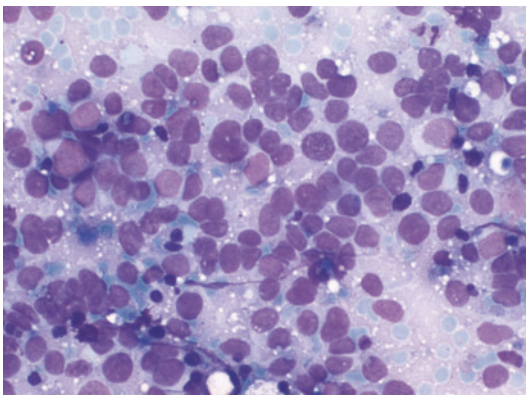
Microscopy

Neuroblastoma: mostly dispersed small, round tumor cells with hyperchromatic nuclei and sparse cytoplasm. Pseudorosettes with central neuropil are often found (Fig. 1).

Ewing sarcoma/PNET: dispersed cells and irregular clusters. Double cell population with small cells with hyperchromatic nuclei and sparse cytoplasm. Larger cells with round nuclei and distinct cytoplasm often with vacuoles. Rosette structures can often be found (Fig. 2).

Desmoplastic round cell tumor: medium-sized tumor cells with round to oval nuclei and scant cytoplasm. Irregular cluster sometimes with epithelial-like structures dominates but dispersed cells are present (Fig. 3).

Alveolar rhabdomyosarcoma: small- to medium-sized monomorphic cells with round to oval nuclei and sparse cytoplasm with few vacuoles. Mostly dispersed cells but alveolar structures are often present. Few cells with rhabdomyoblastic differentiation. Multinucleated tumor cells found in most cases (Fig. 4).

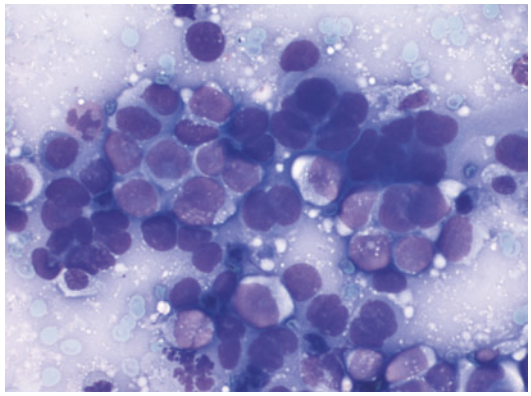


Round Cell Sarcomas, Cytological Findings, Fig. 3 Desmoplastic small-round-cell tumor: rounded tumor cells with scant cytoplasm with a tendency to form small clusters. MGG

Immunophenotype

Neuroblastoma: vimentin weekly positive, synaptophysin, NSE, and NB84 are often positive.

Ewing sarcoma/PNET: vimentin, CD99, and often synaptophysin positive.



Round Cell Sarcomas, Cytological Findings, Fig. 4 Alveolar rhabdomyosarcoma: round tumor cells with monomorphic nuclei often peripherally oriented. The cytoplasm is often rich, *gray-blue* with vacuoles. Cell clusters and alveolar structures can always be found. MGG

Desmoplastic small-round-cell tumor: vimentin, cytokeratin, EMA, and desmin positive.

Alveolar rhabdomyosarcoma: vimentin, desmin, actin, and MyoD1 positive.

Molecular Features

Neuroblastoma: 1p deletion and/or MYCN gene amplification in some cases

Ewing sarcoma/PNET: t(11;22), t(21;22)

Desmoplastic small-round-cell tumor: t(11;22)

Alveolar rhabdomyosarcoma: t(2;13)

Differential Diagnosis

Precursor leukemia/lymphoma, Wilms' tumor, embryonal rhabdomyosarcoma, various round cell sarcoma, melanoma.

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S

Schwannoma, Cytological Findings

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Synonyms

Neurilemoma; Neurinoma

Definition

Benign neoplasia composed of well-differentiated Schwann cells.

Clinical Features

- **Incidence**
  Schwannoma corresponds to 8% of intracranial tumors and 85% of tumors in the region of cerebellopontine angle.
- **Age**
  The schwannoma has a peak of incidence between four to six decades.
- **Sex**
  Most studies show no gender predilection.
- **Site**
  The majority of intracranial lesions involving the eighth cranial nerve in the region of the angle cerebellopontine.
- **Treatment**
  The treatment of patients with schwannoma is surgery.
- **Outcome**
  The lesion is benign, slowly growing; the recurrence is infrequent and rarely occurring malignant changes.

Macroscopy

Schwannoma presents well-circumscribed masses usually bounded by a thick fibrous capsule. The cut surface reveals light tan glistening tissues with yellow patches with or without cysts and hemorrhage.

Microscopy

In cytology, smears are characterized by twisted structures sheaved of closely packed spindle cells with axial orientation (Fig. 1). The cell nuclei may be ovoid or elongated and cigar shaped with rounded ends. They usually have uniform

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
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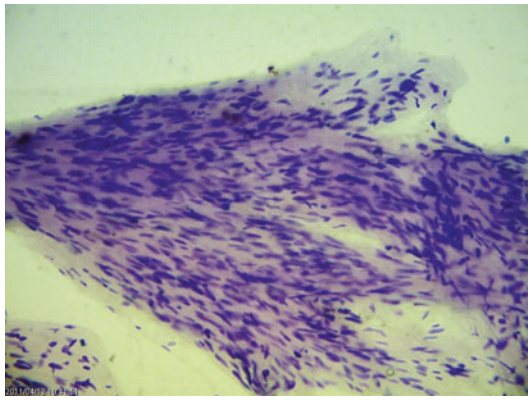
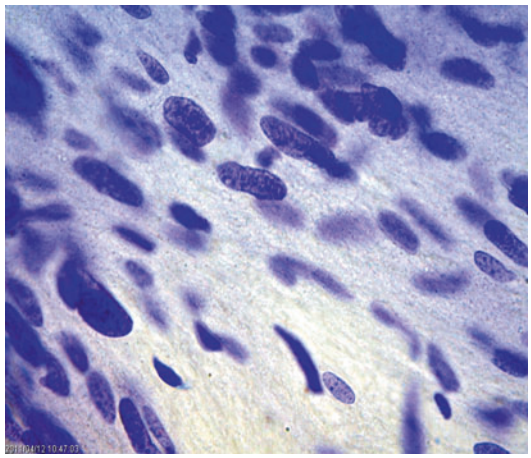


Schwannoma, Cytological Findings, Fig. 1 Schwannoma – note twisted structures sheaved of closely packed spindle cells with axial orientation (*toluidine blue*). Note: Source of the figures of the authors



Schwannoma, Cytological Findings, Fig. 2 Schwannoma – note cells with cytoplasm indistinct, nuclei ovoid or elongated with rounded ends and uniform chromatin (*toluidine blue*). Note: Source of the figures of the authors

chromatin without prominent nucleolus. Cytoplasm is often indistinct, giving the impression of coarsely background stroma (Fig. 2). Ancient schwannomas may show nuclear atypia.

Immunophenotype

The immunophenotype of schwannoma includes reactivities for S-100 protein, Leu-7, and calretinin.

Molecular Features

The molecular alteration observed in schwannoma is inactivating mutation of NF2 gene detected in approximately 60% of the lesions.

Differential Diagnosis

The main differential diagnoses in the region of cerebellopontine angle are meningiomas and metastases. Meningiomas may be identified by their whorls and vesicular nuclei. Metastases of carcinomas usually show atypical and cohesive cells; nuclei demonstrate irregular and coarse chromatin.

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Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings

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Definition

Sebaceous carcinoma is found almost exclusively in the eyelid skin and arises from the meibomian gland or other local sebaceous glands.

Clinical Features

- **Incidence**
  1–5% of eyelid malignancies.
- **Age**
  Old age.
- **Sex**
  No gender predilection.
- **Site**
  Eyelid skin, higher incidence in the upper eyelid.
- **Treatment**
  Surgery, chemotherapy, and radiotherapy.
- **Outcome**
  Sebaceous carcinomas may involve adjacent structures or metastasize to cervical or preauricular lymph nodes.

Macroscopy

Clinically, they present as firm, subcutaneous nodules resembling a chalazion or other lesions, which results in delayed diagnosis and relatively high morbidity and mortality. Sometimes the tumor growth results in a pagetoid or carcinoma in situ-like involvement of the conjunctiva causing persistent unilateral conjunctivitis before the correct diagnosis is made.

Microscopy

The tumor has a lobular pattern and is formed by cells with variable sebaceous differentiation depending on their grade of differentiation. FNA shows hyperchromatic nuclei, prominent nucleoli, and abundant cytoplasm which contains small lipidic vacuoles. FNA of lymph node metastases from sebaceous carcinomas has also been described.

Differential Diagnosis

Congenital sebaceous gland hyperplasia, adenoma sebaceum

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Secretory Carcinoma, Cytological Findings

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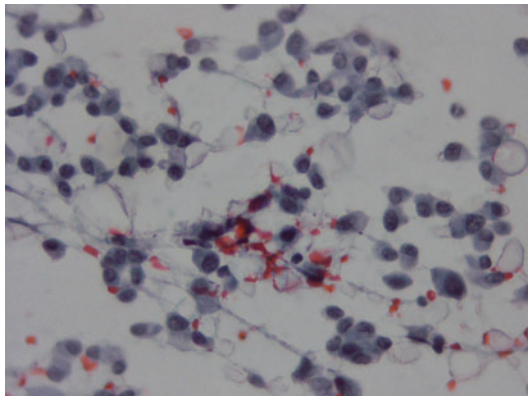
Synonyms

Juvenile breast carcinoma



Secretory Carcinoma, Cytological Findings, Fig. 1 Secretory carcinoma. Note dispersed cells with vacuolated cytoplasm (Papanicolaou staining)

Definition

It is a rare low-grade variant of breast cancer, translocation associated, with a solid, microcystic, and tubular architecture composed of cells that produce intracellular and extracellular secretory material (Fig. 1).

Clinical Features

- **Incidence**
  Extremely rare (less than 0.15% of all breast cancers).
- **Age**
  It is the most common breast carcinoma under the age of 30, and it is the most common type of breast carcinoma in children. The median age of presentation is 25 years.
- **Sex**
  Occurs in both sexes with a predominance in females.
- **Site**
  Usually subareolar.
- **Treatment**
  Surgery in the form of mastectomy with axillary clearance is the treatment of choice.
- **Outcome**
  Local recurrence after a long disease-free interval has been described mostly in patients that underwent conservative surgery. Distant metastases from secretory carcinoma are extremely rare.

Macroscopy

The most frequent clinical presentation is of an asymptomatic mobile mass, which is usually subareolar. The tumor size varies from 1 to 16 cm with an average diameter of 3 cm.

Microscopy

Cytological features revealed mildly atypical cells organized in sheets or groups in a background of thyroid similar material. The cells show a characteristically vacuolated cytoplasm, with the majority having mostly multiple small vacuoles, but occasional cases had prominent monovacuolated cells, some of which resembled signet ring cells. Some of these vacuoles contained mucin, as evidenced on special stains. The cytoplasm of the tumor cells is finely granular and very eosinophilic on hematoxylin- and eosin-stained smears.

Histologically, secretory carcinomas can demonstrate several histological patterns including solid, microcystic, and ductal, with many tumors containing all three patterns. The tumor cells are polygonal with granular eosinophilic cytoplasm, with intracellular and extracellular PAS- and alcian-blue-positive secretions. Atypia is minimal or absent and mitotic activity is low.

Immunophenotype

Epithelial membrane antigen (EMA), S-100 protein, and alpha-lactalbumin are frequently positive. CK 8/18 and E-cadherin are usually positive. ER, PR, and HER2 are negative.

Molecular Features

The tumor is the only epithelial tumor of the breast with a balanced translocation, t(12;15), that creates an ETV6-NTRK3 gene translocation. The consequence of this translocation is the fusion of the dimerization domain of a transcriptional regulator (ETV6) with a membrane receptor tyrosine

kinase (NTRK3) that activates the Ras-Mek1 and PI3K-Akt pathways which are important for breast cell proliferation and survival. This specific translocation is associated with congenital fibrosarcoma and mesoblastic nephroma, two morphologically similar pediatric mesenchymal tumors with no epithelial features.

Differential Diagnosis

The main important differential diagnoses are represented by benign epithelia proliferative lesions such as lactational changes/lactating adenoma. In the field of malignant lesions, lipid-secreting carcinoma and acinic cell carcinoma must be considered.

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Serous Ovarian Tumors, Cytological Findings

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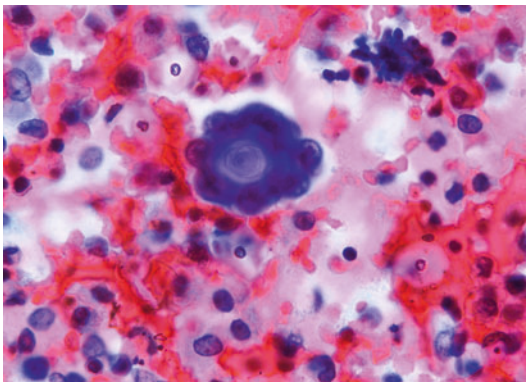
Synonyms

A typical proliferative serous tumor of ovary; Benign serous tumors; Serous adenocarcinoma; Serous cyst; Serous cystadenoma or adenofibroma; Serous cystoma; Serous ovarian tumor of low malignant potential; Serous tumor of borderline malignancy

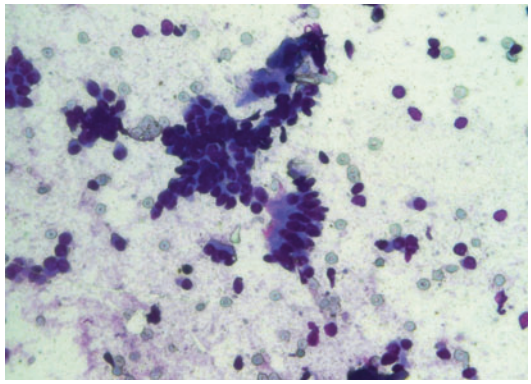
Microscopy

Benign Serous Tumors: Cytologic Findings

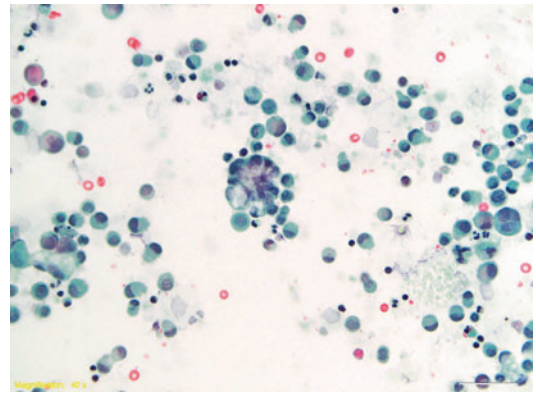
- **Fluid Aspiration (FNA)**. Macroscopically, it is usually clear (Sudha 2011). Microscopically, cellularity is generally low, with small round to cuboidal cells that are dispersed or forming small clusters or honeycomb sheets (Fig. 1). Cytoplasm is scant and pale; nuclei are uniform and round containing pale chromatin. Cilia are sometimes present (Ivić 1976). Fluid usually contains some phagocytes and ciliocytophtoria (Ivić 1976).
- **Imprint**. Cellularity is generally moderate with small round to cuboidal cells forming sheets and clusters. Cytoplasm is scant and pale; nuclei are uniform and round containing pale chromatin. Cilia are rarely present (Fig. 2). Stromal cells can be seen in variable amount. A pattern of branching sheets and clusters with a lot of bare round to elongated nuclei in background indicates cystadenofibroma (Ivić 1976) (Fig. 3).
- **Peritoneal washing**. Usually does not contain epithelial cells.



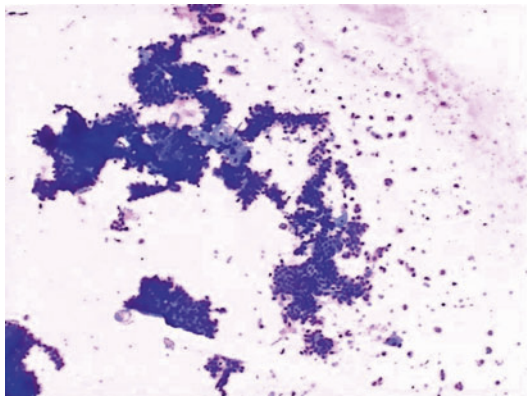
Serous Ovarian Tumors, Cytological Findings, Fig. 1 Benign serous tumor. Cluster of small cells containing psammoma body (Cyst aspirate, MGG, ×1,000)



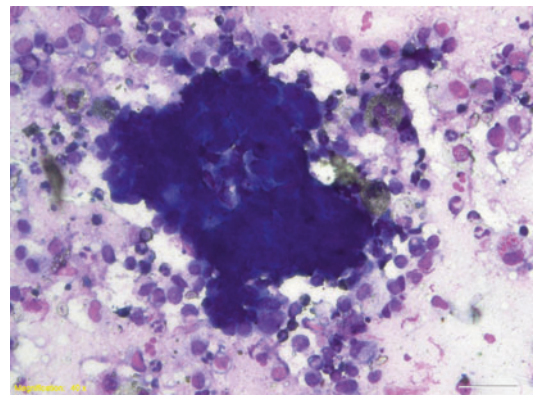
Serous Ovarian Tumors, Cytological Findings, Fig. 2 Benign serous tumor. Clusters and sheets of small round to cuboidal cells, some containing cilia on the top (Imprint, MGG, ×400)



Serous Ovarian Tumors, Cytological Findings, Fig. 4 Serous borderline tumor. Papilla-like formation of round to cuboidal, medium sized cells with cytoplasmic vacuoles. Nuclei are mildly pleomorphic (Cyst aspirate, Papanicolaou, ×400)



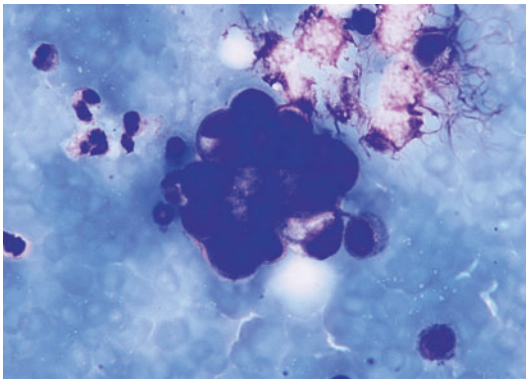
Serous Ovarian Tumors, Cytological Findings, Fig. 3 Cystadenofibroma. Branching clusters and sheets of small round to cuboidal cells, a lot of bare nuclei in the background (Imprint, MGG, ×100)



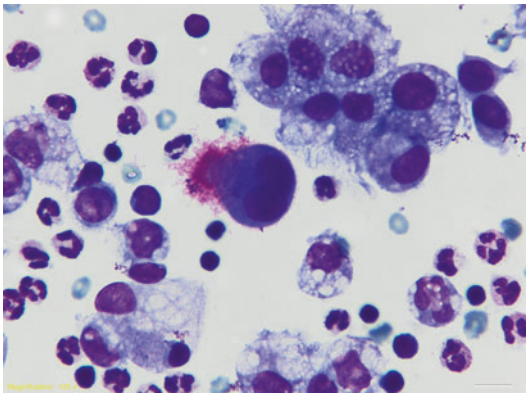
Serous Ovarian Tumors, Cytological Findings, Fig. 5 Serous borderline tumor. There is some anisocytosis and anisonucleosis, chromatin is rougher, slightly irregular (Imprint, MGG, ×400)

Serous Borderline Tumors (SBOT): Cytologic Findings

- **Fluid Aspiration (FNA)**. Macroscopically, fluid is usually clear to turbid (Sudha 2011). Microscopically, cellularity is moderate to high with cells that are round to cuboidal, medium sized. Cells can be arranged in small clusters with an acinar pattern or papilla-like formations (Fig. 4). Single cells are rare. Nuclei are mildly pleomorphic, chromatin is irregular, and mitoses are rare. Cytoplasmic vacuoles can be seen. Usually, there is a presence of benign epithelium. Ebner cells and psammoma bodies can be seen. Fluid usually contains phagocytes. Ciliocytophtoria can be seen (Ivić 1976).
- **Imprint**. Cellularity is moderate to abundant. Cells are arranged in groups, papillary formations, palisades, and individually (Fig. 5). Signs of malignancy are not so prominent. There is slight anisocytosis and anisonucleosis, chromatin is rougher, slightly irregular with prominent nucleoli. Mitoses are rare. Stromal



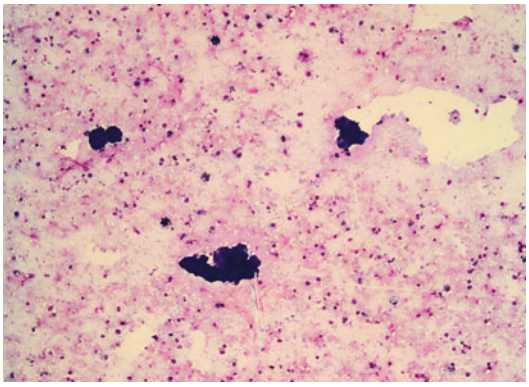
Serous Ovarian Tumors, Cytological Findings, Fig. 6 Serous borderline tumor. Papilla-like formation of epithelial cells with mildly pleomorphic nuclei and irregular chromatin (Peritoneal washing, MGG, ×1,000)



Serous Ovarian Tumors, Cytological Findings, Fig. 7 Serous borderline tumor. Ciliated (Ebner) cell among mesothelial and phagocytes (Peritoneal washing, MGG, ×1,000)

cells can be seen in variable amount (Khunamornpong and Siriaunkgul 2003; Ivić 1976).

- **Peritoneal washing**. Presence of epithelial cells depends on progression of disease (peritoneal implants) and presence of papillary growths springing from the surface (about 30% of all SBOT) (Tavaddoli and Devilee 2003). Morphologically, cells are similar to those in cyst aspirate (Fig. 6), but more degenerated. Ciliocytophtoria, Ebner cells (Fig. 7), and psammoma bodies can be seen.

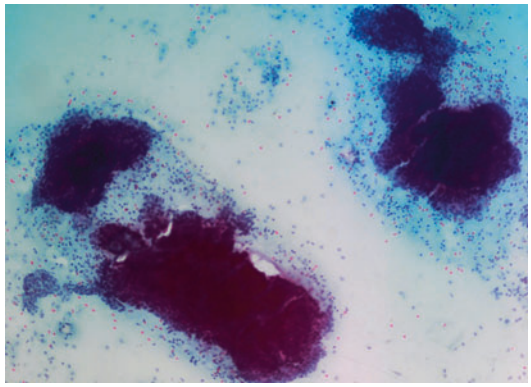


Serous Ovarian Tumors, Cytological Findings, Fig. 8 Micropapillary serous carcinoma. Tumor cells are arranged in 3-dimensional clusters and papillary groups (Cyst aspirate, MGG, ×100)

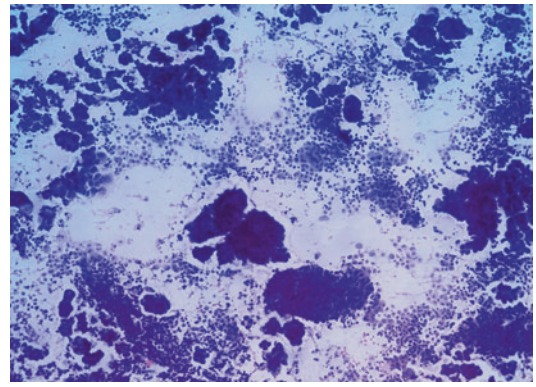
Malignant Serous Tumors: Cytologic Findings

- **Fluid Aspiration (FNA)**. Macroscopically is usually turbid, sometimes slightly reddish. Microscopically, cellularity is usually abundant. The tumor cells are arranged in 3-dimensional clusters and papillary groups (Fig. 8). Individual cells are more often present in poorly differentiated carcinoma. Epithelial cells are low columnar, chromatin is mostly irregular, nucleoli are multiplied and prominent, mitoses are not rare. Sometimes cannibalism can be seen. Ebner cells and psammoma bodies can be helpful in differential diagnosis (Ivić 1976). Phagocytes are usually present in variable number.
- **Imprint**. Differentiation between well-differentiated serous carcinoma and borderline tumors is difficult, if not impossible (Sudha 2011). Cellularity is usually abundant (Fig. 9). Cells are arranged in groups, papillary formations, and palisades where nuclei are overlapping. There are some single malignant cells and bare malignant nuclei. Cytoplasm can be slightly vacuolated. Chromatin is irregular; nucleoli are multiplied and more prominent.

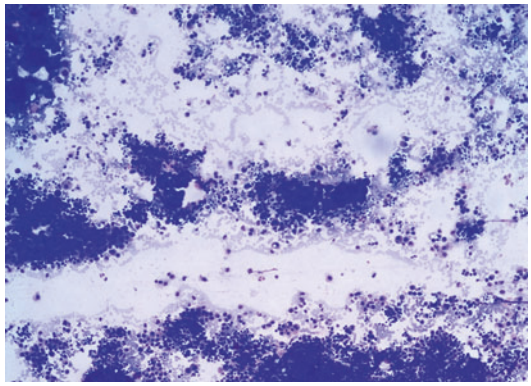
  Cells in poorly differentiated carcinoma form groups or they are single. There are no papillary formations. Anisocytosis, anisonucleosis, polymorphism, and multinucleation are well-marked. Nuclei are big with common overlapping. Chromatin is irregular, lumpy, but



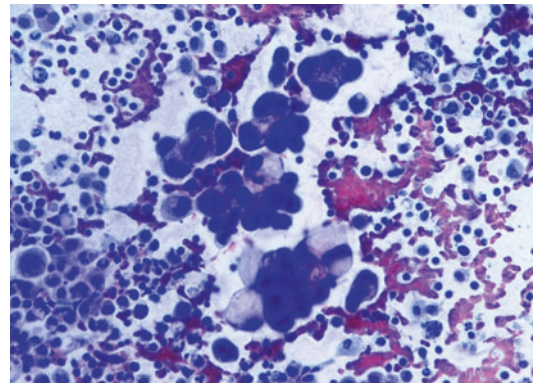
Serous Ovarian Tumors, Cytological Findings, Fig. 9 Carcinoma papillary gr I. Abundant cellularity, cells are arranged in groups, papillary formations, and palisades (Imprint, Papanicolaou, ×100)



Serous Ovarian Tumors, Cytological Findings, Fig. 11 Serous carcinoma. Abundant cellularity, clusters, papillary formations, and single malignant cells (Ascites, Papanicolaou, ×100)



Serous Ovarian Tumors, Cytological Findings, Fig. 10 Serous carcinoma. Abundant cellularity, clusters, papillary formations, and single malignant cells (Ascites, MGG, ×100)



Serous Ovarian Tumors, Cytological Findings, Fig. 12 Serous carcinoma. Papillary formations of tumor cells with cytoplasmic vacuolization and mitoses (Ascites, Papanicolaou, ×400)

it can be immature. Nucleoli are usually very prominent, sometimes big irregular in shapes. Cytoplasms are deficient or absent, so background can be formed of decomposed cytoplasm with fine vacuolization.

- **Peritoneal washing: Ascites**. Cells from peritoneal (pleural) metastases desquamate mainly in papillary formations, clusters, and acinar formations (Figs. 10–12). Single cells and bizarre cells are more often in poorly differentiated carcinoma. Cytoplasmic vacuolization is more common than in cyst aspirate and signet ring cells are more frequent. Nucleoli are prominent and mitoses are frequent (Fig. 12). Ebner cells and psammoma bodies can be seen (Ivić 1976). Background tends to be hemorrhagic and necrotic.

Immunophenotype

Serous carcinoma cells are positive to CK7, EMA, Ber EP4, CA 125 (85%) (Tavaddoli and Devilee 2003) and are negative to CK 20, calretinin, and other mesothelial markers (Sudha 2011).

Differential Diagnosis

Ciliated (Ebner) cells indicate serous origin but do not have diagnostic significance. Psammoma bodies can be found in serous, but also in endometrioid carcinoma (Ivić 1976).

Benign epithelial inclusions in peritoneum: small round glands lined by a single layer of flat to low columnar cells without atypia.

Endosalpingiosis can have papillary growth but is lined by a single layer of cuboidal epithelium. Complex papillary growth, some degree of cytologic atypia, tufting, and stratification are requirements for a diagnosis of extraovarian borderline tumor. Lesions that have some but not all features of SBOT have been designated as atypical endosalpingiosis (Acs 2005).

Serous tumors of low malignant potential can be mistaken for both benign cystadenomas (absence of complex branching, nuclear pleomorphism, and hyperchromasia) and well-differentiated carcinomas (presence of stromal invasion cannot be seen cytologically). Any loss of papillae, dyshesion, or irregular group contours excludes an SBOT diagnosis (Weir and Bell 2001).

Differentiation from reactive mesothelial cells is a common problem. A general diagnostic approach suggested by Weir and Bell is the following: A diagnosis of reactive mesothelial cells can be made when a specimen shows cohesive cell groups with irregular contours, intercellular windows, no papillae, mild nuclear atypia, minimal nuclear overlap, low N/C ratios, small nuclei, and small cells (Weir and Bell 2001).

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Sex Cord-Stromal Tumors, Cytological Findings

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Synonyms

Ovarian stromal cell tumors; Sex cord tumors

Definition

Sex cord-stromal tumors (SCST) are included in category of ovarian neoplasms derived from the granulosa cells, theca cells, and their luteinized variants. These tumors can be of different morphological types and of various stages of differentiation.

Classification of SCST:

1. Granulosa stromal cell tumors
   (a) Granulosa cell tumors
   (b) Tumors in the thecoma-fibroma group
2. Sertoli-stromal cell tumors
3. Gynandroblastoma
4. Sex cord tumor with annular tubules

5. Unclassified

Sertoli-stromal tumors can occur in the ovary and in the testis, being more common in the testis in morphological variants of pure Sertoli, pure Leydig, and combined Sertoli-Leydig tumor (Young and Scully 2002).

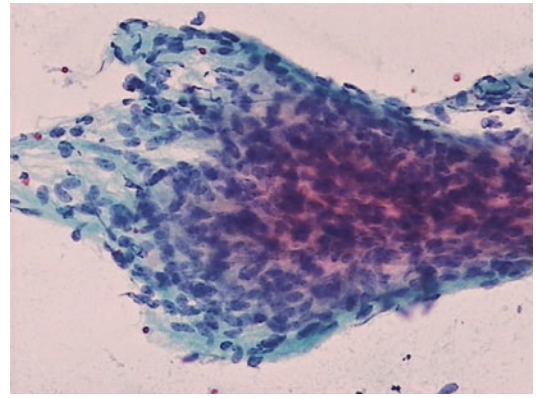
Microscopy

Cytological features of the SCST, except for the granulosa cell tumors, are sparsely described in the literature. Cytological techniques of acquiring cytological sample are fine needle aspiration and tumor imprint or scraping during the operative treatment. Implementation of cytology in diagnosis of SCST can be in preoperative evaluation of ovarian tumors combined with other diagnostic procedures such as ultrasound and serum tumor markers. Recently, FNA of ovarian tumors were mostly abandoned due to possible spread of tumor cells in the case of malignant lesion. In intraoperative evaluation of cytological imprint and/or scraping with rapid staining could be combined with intraoperative frozen section diagnosis of ovarian neoplasms to obtain the most precise intraoperative diagnosis. Cytologist, evaluating intraoperative imprints of SCST, must be highly experienced in analyzing cytomorphology of ovarian neoplasms and be aware of the limitations of the procedure (Acs 2002; Gupta and Baloch 2002; Khunamornpong and Siriaunkgul 2003).

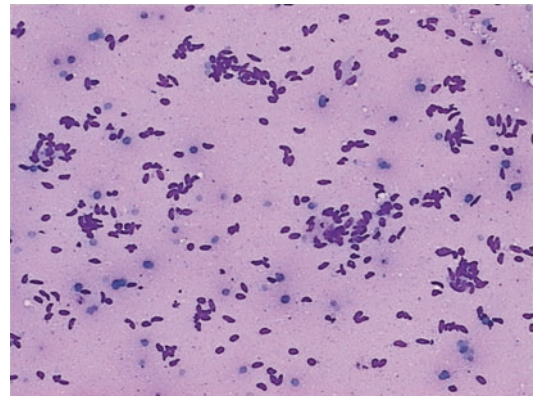
Thecoma-Fibroma Tumor

Imprint Cytology

Moderately cellular imprints containing tissue fragments of uniform spindle cells. Cytoplasms are pale basophilic, elongated with confluent borders. Nuclei are elongated and spindle with regular, fine chromatin. Many elongated and spindle bare nuclei of the same morphology as in tissue fragments could be seen. In the tumors with mainly thecoma component, bare nuclei are more plump. Background is clean, sometimes eosinophilic, and granular especially in thecoma type of tumors (Figs. 1 and 2, source: slide archive of the Department of Clinical Cytology, Clinical Hospital Centre Rijeka, Croatia).



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 1 Thecoma-fibroma tumor. Tissue fragment of uniform spindle cells (Imprint, Papanicolaou ×100)

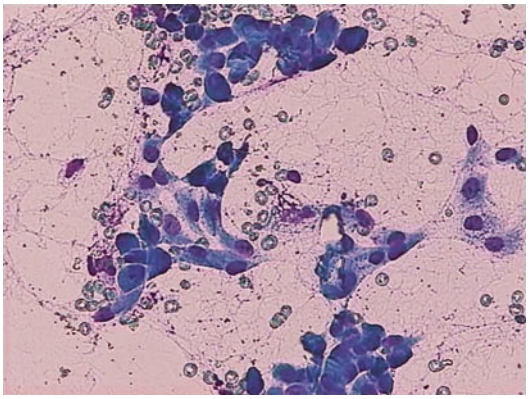


Sex Cord-Stromal Tumors, Cytological Findings, Fig. 2 Thecoma-fibroma tumor. Bare spindle nuclei (Imprint, MGG ×100)

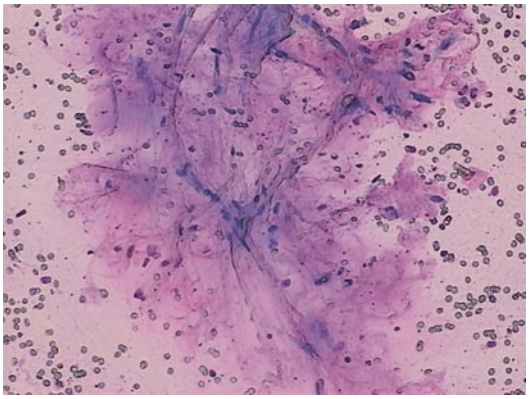
Sclerosing Stromal Tumor of the Ovary

Imprint Cytology

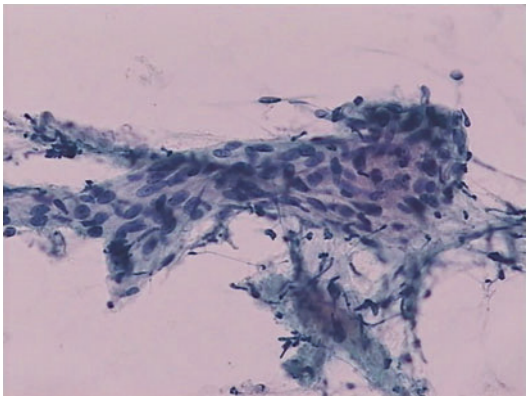
Cellularity of the imprint is low. There are few small clusters of uniform polygonal "epithelioid" cells. Tissue fragments are intermediate or hipocellular with few uniform spindle cells and abundant fine filamentous extracellular substance. Occasional spindle bare nuclei are seen in the overall clean background (Mikami et al. 2003) (Figs. 3–5, source: slide archive of the Department



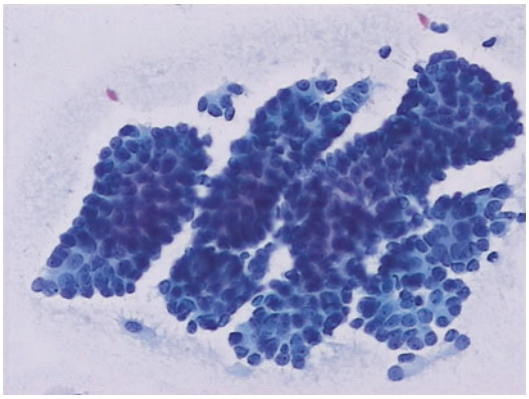
Sex Cord-Stromal Tumors, Cytological Findings, Fig. 3 Sclerosing stromal tumor of the ovary. Clusters of uniform polygonal "epithelioid" cells (Imprint, MGG ×200)



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 5 Sclerosing stromal tumor of the ovary. Hipocellular tissue fragment with few uniform spindle cells and abundant fine filamentous extracellular substance (Imprint, MGG ×100)



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 4 Sclerosing stromal tumor of the ovary. Tissue fragments with uniform spindle cells (Imprint, Papanicolaou ×200)



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 6 Sertoli-Leydig tumor. Tubular clusters of polygonal Sertoli-type cells with rosette formations (Imprint, Papanicolaou ×400)

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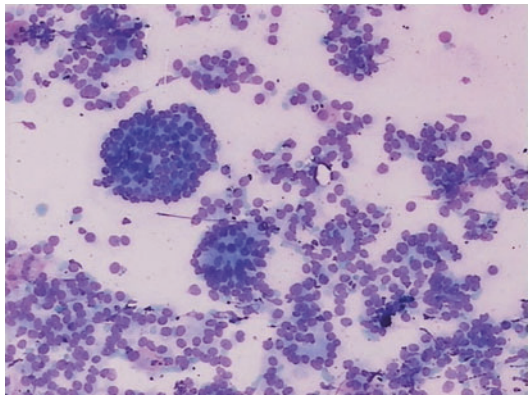
of Clinical Cytology, Clinical Hospital Centre Rijeka, Croatia).

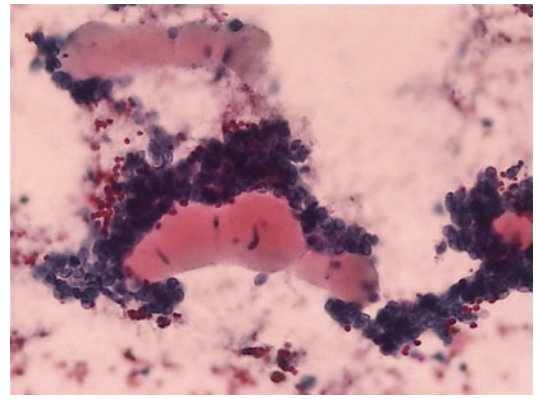
Sertoli-Stromal Cell Tumors

Imprint Cytology

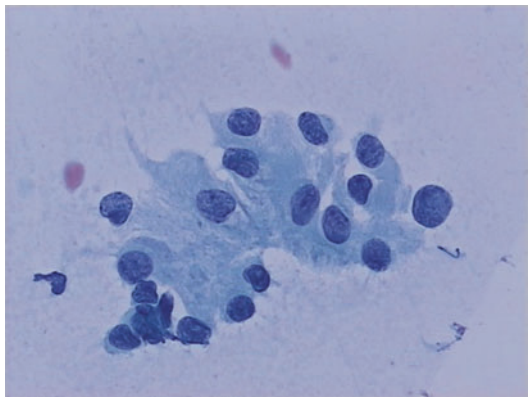
Hypercellular imprints with monomorphic cuboid and low columnar cells with epithelioid appearance. Nuclei are round with fine chromatin. Cytoplasms are basophilic, moderately abundant, or scant with well-defined borders. Cells are mainly arranged in tubular clusters or small aggregates with occasional rosette formations. In the case of Sertoli-Leydig tumor variant, along with previously described Sertoli type of cells, Leydig cells could be identified as large, polygonal cells with round nuclei, fine chromatin, and pale blue ill-defined large cytoplasm arranged in small and loose clusters (Figs. 6–8, source: slide archive of the Department of Clinical Cytology, Clinical Hospital Centre Rijeka, Croatia).



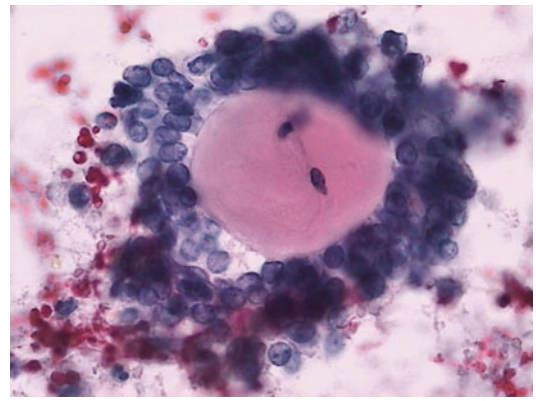
Sex Cord-Stromal Tumors, Cytological Findings, Fig. 7 Sertoli-Leydig tumor. Clusters and small aggregates of uniform Sertoli-type cells with rosette formations (Imprint, MGG ×100)



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 9 Sex cord tumor with annular tubules. Extracellular, globular, eosinophilic substance attached to the tubular cluster of small uniform cuboid cells (Imprint, Papanicolaou ×100)



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 8 Sertoli-Leydig tumor. Leydig cells (Imprint, Papanicolaou ×400)



Sex Cord-Stromal Tumors, Cytological Findings, Fig. 10 Sex cord tumor with annular tubules. Extracellular, globular, eosinophilic substance in the center of the cluster of small uniform cuboid cells (Imprint, Papanicolaou ×400)

Sex Cord Tumor with Annular Tubules

Imprint Cytology

Moderately cellular imprints with monomorphic, small cuboid, or low columnar cells. Nuclei are round with fine chromatin and may be hypochromatic. Cytoplasms are light, basophilic, and scant with ill-defined borders. Cells are mainly arranged in solid and tubular clusters or loose aggregates with extracellular, homogenous, globular, eosinophilic, or metachromatic substance attached to or in the center of the cluster (Premalata et al. 2005) (Figs. 9 and 10, source: slide archive of the Department of Clinical Cytology, Clinical Hospital Centre Rijeka, Croatia).

Fine Needle Aspiration Cytology of Sex Cord-Stromal Tumors

Fine needle aspiration procedures of the ovarian tumors are rarely performed, but it could be assumed that microscopic pattern is similar to imprint cytology but cellularity could be lower.

Peritoneal Fluid

Ascitic fluid could be present in some cases of sex cord-stromal tumors. These tumors are mainly benign, so ascitic fluid is mainly a consequence of reactive changes of peritoneal lining and has nonspecific microscopic pattern. In those cases ascitic fluid contains reactive mesothelial cells, macrophages, histiocytes, and few lymphocytes without identifiable tumor cells.

Differential Diagnosis

Differential diagnosis for thecoma-fibroma tumors, other benign mesenchymal tumors; for Sertoli or Sertoli-Leydig tumors, epithelial ovarian tumors especially endometrioid, granulosa cell tumor, and carcinoid; and for sex cord tumor with annular tubules, clear cell carcinoma of the ovary and adenoid-cystic tumor (Young and Scully 2002).

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Small Cell Carcinoma, Cytological Findings

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Definition

Small cell lung carcinoma (SCLC) is an aggressive, high-grade neuroendocrine carcinoma occurring in cigarette smokers. The presenting symptoms are usually nonspecific like cough, hemoptysis, and weight loss or can be due to paraneoplastic syndromes associated with SCLC.

Clinical Features

- **Incidence**
  SCLC accounts for approximately 15% of all lung cancers.
- **Age**
  Most are older patients over 60 years of age.
- **Sex**
  SCLC used to be much more common in men, but with the change in smoking habits, the proportion of women with SCLC increased to 50% in the United States.
- **Site**
  Most SCLC are central with extensive spread to hilar and mediastinal lymph nodes. Peripheral SCLC presenting as a solitary pulmonary nodule are rare. Two-thirds of patients with SCLC have extensive disease and over 50% of patients have already distant metastases at time of diagnosis.
- **Treatment**
  SCLC are typically unresectable and treated with combination chemotherapy and radiation.
- **Outcome**
  The prognosis of SCLC remains very poor with a 5-year survival rate of only 5%.

Macroscopy

Most SCLC present as a large central mass involving mediastinal lymph nodes.

Microscopy

SCLC, as the name implies, is composed of small cells that usually measure two to three times the size of a lymphocyte (Fig. 1). But in well-fixed preparations and effusions (though they rarely affect the pleura), the cell size can be significantly larger (Fig. 2). Nuclear features and the scant cytoplasm with ill-defined cell borders are the key diagnostic criteria for SCLC. The nuclei have finely and evenly dispersed chromatin with inconspicuous or undetectable nucleoli. In poorly preserved cells the nuclei are more hyperchromatic.

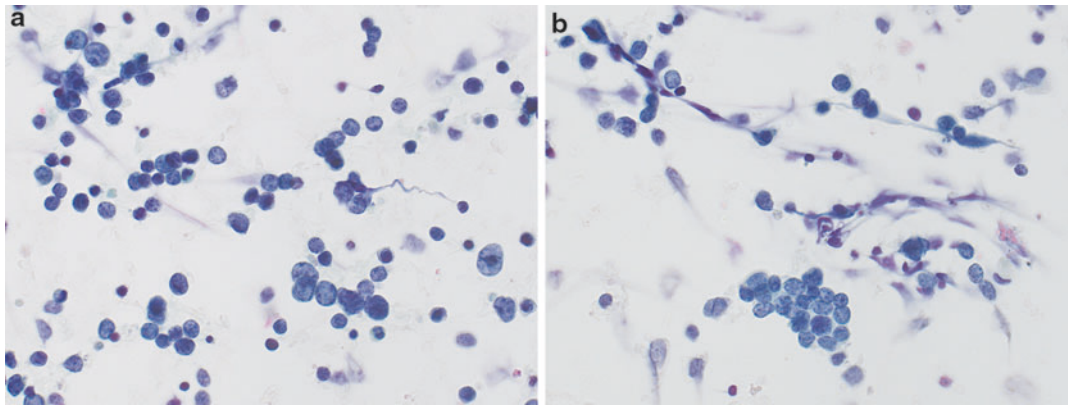
The cells are round to oval or spindle-shaped with a high nuclear to cytoplasmic ratio and are arranged in sheets and aggregates, single files, or as individual single cells. Nuclear molding and crush artifacts are characteristic, but not specific for SCLC. Due to their small size, the cells can appear uniform, but at closer inspection they are clearly polymorphic and show mitoses. Necrosis is commonly present.

Immunophenotype

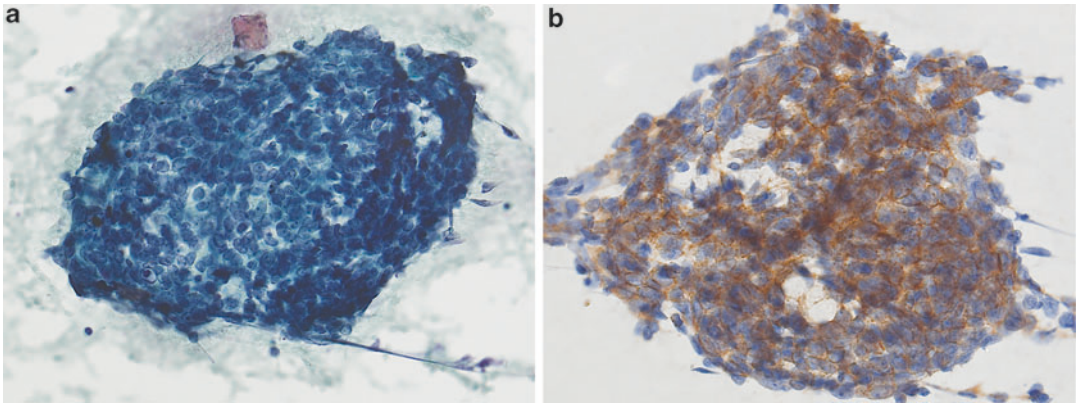
Most SCLC can be readily diagnosed by morphology alone, but immunostains are very helpful in challenging cases. The most reliable neuroendocrine marker in SCLC is CD 56, which should be used in a panel together with chromogranin and synaptophysin. 10% of SCLC are negative for all neuroendocrine makers, which does not preclude the diagnosis. Almost all SCLC express cytokeratin and 70–80% express TTF-1. TTF-1 can be positive in extrapulmonary small cell carcinomas and is therefore not specific for a pulmonary primary. The Ki-67 labeling index is high (>50%).

Differential Diagnosis

The treatment-relevant distinction between SCLC and non-small cell lung cancer (NSCLC) is based



Small Cell Carcinoma, Cytological Findings, Fig. 1 (**a**) Carcinoma cells arranged as individual single cells and single files with nuclear molding. The cells are small but polymorphic with scant cytoplasm and ill-defined cell borders. The nuclei have finely and evenly dispersed chromatin with inconspicuous or undetectable nucleoli. Some nuclei are more hyperchromatic. (**b**) In this field the cells form a small, tight aggregate, and the characteristic crush artifacts with smearing of nuclear material are present (Mediastinal lymph node, transbronchial fine needle aspiration. Papanicolaou stain, magnification ×600)



Small Cell Carcinoma, Cytological Findings, Fig. 2 (**a**) In effusions the cell size can be larger than two to three times the size of a lymphocyte, but the nuclear features and the scant cytoplasm with ill-defined cell borders are characteristic for SCLC. In this case the cells are arranged in large, tight clusters. The nucleoli are visible but inconspicuous (Papanicolaou stain, magnification ×600). (**b**) By immunocytochemistry the carcinoma cells express the neuroendocrine marker CD56 (magnification ×600)

on the characteristic nuclear and cytoplasmic features. Large cell neuroendocrine carcinoma (LCNEC) of the lung typically shows cell characteristics of NSCLC with tight cohesive clusters of large polymorphic cells, coarse or vesicular chromatin, and prominent nucleoli. However, a morphologic overlap between SCLC and LCNEC does exist and these cases are designated as high-grade neuroendocrine carcinomas.

Tumors composed of small basaloid cells with high nuclear to cytoplasmic ratio have broad differential diagnoses and are well-known pitfalls in thoracic pathology. In difficult cases careful evaluation of well-preserved and non-traumatized tumor cells, especially if extensive crush artifacts are present, will guide toward decision of the appropriate immunocytochemistry panel and along with appropriate clinical information prevent misinterpretation. The most important differential diagnoses include carcinoid tumors of the lung (see entry on "► Carcinoid Tumors of the Lung, Cytological Findings"), poorly differentiated, basaloid squamous cell carcinoma, and rarely lymphoma, primitive neuroectodermal tumor, melanoma, paraganglioma, and Merkel cell carcinoma.

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Spindle Cell Sarcomas, Cytological Findings

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Variants

Leiomyosarcoma, Malignant peripheral nerve sheet tumor, Monophasic synovial sarcoma, Dermatofibrosarcoma protuberans, Fibrosarcoma (childhood, adult), Spindle cell gastrointestinal stromal tumor (GIST).

Definition

Malignant spindle cell tumors.

Clinical Features

- **Incidence**
  Leiomyosarcoma: 5 per 1,000,000.
  Malignant peripheral nerve sheet tumor: 2 per 1,000,000.
  Monophasic synovial sarcoma: 2–3 per 1,000,000.
  Dermatofibrosarcoma protuberans: 0.8 per 1,000,000.
  Fibrosarcoma (adult): 0.6 per 1,000,000.
  Spindle cell GIST: 10–20per 1,000,000.
- **Age**
  Leiomyosarcoma: middle aged-older patients
  Malignant peripheral nerve sheet tumor: all ages.
  Monophasic synovial sarcoma: most cases before 50 years.
  Dermatofibrosarcoma protuberans: young adults.
  Fibrosarcoma – infantile: early childhood and congenital cases.
  Fibrosarcoma – adults: middle aged and old
  Spindle cell GIST: middle aged.
- **Sex**
  Leiomyosarcoma: retroperitoneal tumors more common in women.
  Malignant peripheral nerve sheet tumor: 5 times more common in males with von Recklinghausen's disease.
  Monophasic synovial sarcoma: more common in men.
  Dermatofibrosarcoma protuberans: slight male predominance.
  Fibrosarcoma – infantile: slight male predominance.
  Fibrosarcoma – adult: no sex predilections
  Spindle cell GIST: slightly more common in men.
- **Site**
  Leiomyosarcoma: retroperitoneum, large vessels, extremities (deep or superficial).
  Malignant peripheral nerve sheet tumor: proximal extremities, brachial plexus, paraspinal nerves.
  Monophasic synovial sarcoma: deep soft tissue of extremities.
  Dermatofibrosarcoma protuberans: dermis and subcutis of limbs and trunk.
  Fibrosarcoma – infantile: deep soft tissue of extremities and trunk.
  Fibrosarcoma – adult.
  Spindle cell GIST: gastrointestinal tract, most common in the stomach and small intestine.
- **Treatment**
  Surgery, radiotherapy, chemotherapy.
- **Outcome**
  Leiomyosarcoma: retroperitoneal and large vessel tumors are often fatal.
  Malignant peripheral nerve sheet tumor: high-grade tumors have poor prognosis.
  Monophasic synovial sarcoma: approximately 50% survive.
  Dermatofibrosarcoma protuberans: most patients survive but recurrences are seen in approximately 40% of cases.
  Fibrosarcoma – infantile: approximately 80% survive.
  Fibrosarcoma – adult: approximately 45% survive.
  Spindle cell GIST: 45% 5 years survival.

Microscopy

Leiomyosarcoma: dispersed and aggregates of pleomorphic spindle cells with elongated blunt-ended nuclei which often are truncated (Fig. 1).

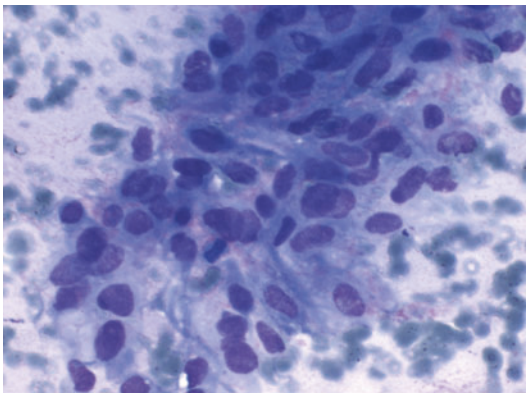
Malignant peripheral nerve sheet tumor: elongated tumor cells with thin cytoplasm and elongated nuclei. Dispersed cells and clusters. Fibrillar background in clusters (Fig. 2).

Monophasic synovial sarcoma: uniform oval tumor cells with poorly defined cytoplasm. Stripped nuclei with inconspicuous nuclei. Some tissue fragments with packed cells (Fig. 3).

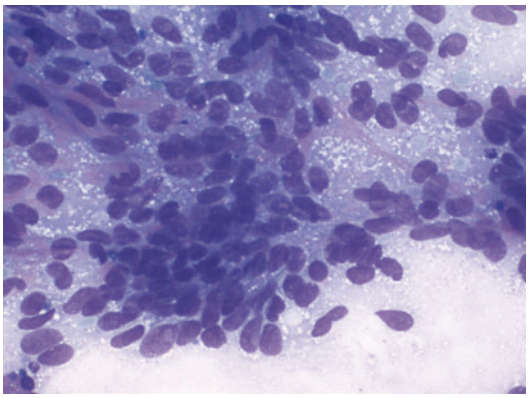
Dermatofibrosarcoma protuberans: relatively dispersed spindle cells with moderate variation in nuclear size and shape. Sparse pale cytoplasm. Few dense clusters (Fig. 4).

Fibrosarcoma – infantile: uniform spindle cells with bland nuclei and poorly defined bipolar cytoplasm (Fig. 5a).

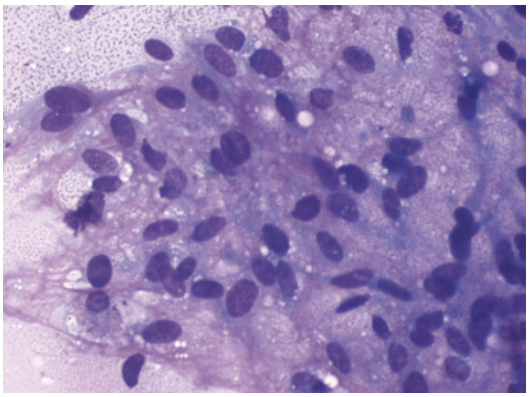
Fibrosarcoma – adult: relatively uniform spindle cells with hyperchromatic fusiform nuclei. Fascicular structures often present (Fig. 5b).



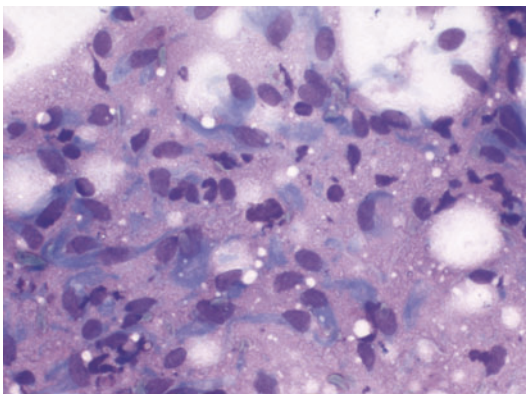
Spindle Cell Sarcomas, Cytological Findings, Fig. 1 Leiomyosarcoma: spindle cells with oval to round nuclei and elongated *gray-blue* cytoplasm. The cells are often held together in irregular fragments. MGG



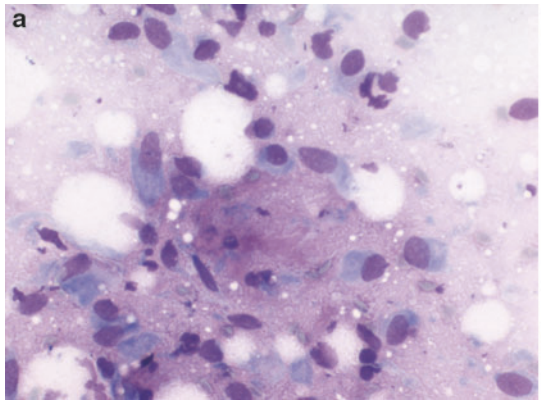
Spindle Cell Sarcomas, Cytological Findings, Fig. 3 Monophasic synovial sarcoma: oval- to spindle-shaped tumor nuclei with poorly defined cytoplasm. Tight cell clusters are often present. MGG

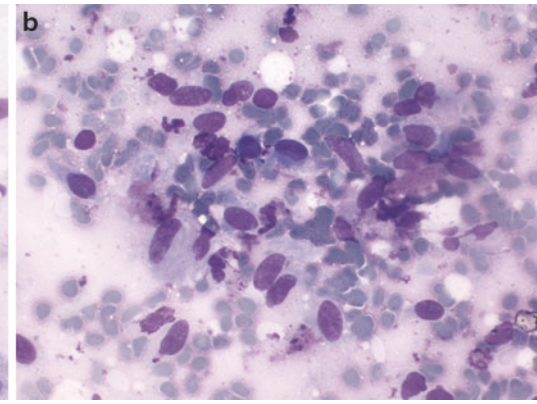


Spindle Cell Sarcomas, Cytological Findings, Fig. 2 Malignant peripheral nerve sheet tumor: spindle tumor cells with oval nuclei and elongated cytoplasm in a loose network of fibrillar substance. MGG

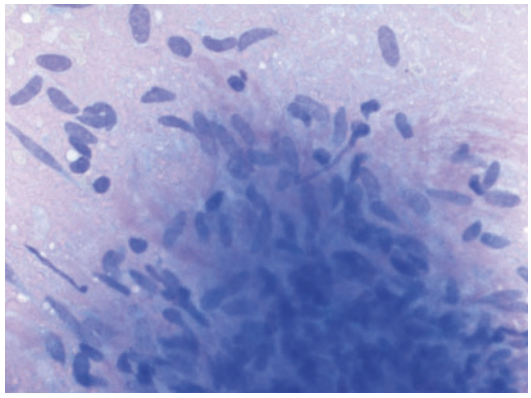


Spindle Cell Sarcomas, Cytological Findings, Fig. 4 Dermatofibrosarcoma protuberans: moderately pleomorphic tumor cells with irregular hyperchromatic nuclei and poorly defined cytoplasm. MGG





Spindle Cell Sarcomas, Cytological Findings, Fig. 5 (**a**) Fibrosarcoma – infantile: fibroblast-like tumor cells with hyperchromatic oval nuclei and distinct elongated cytoplasm. MGG. (**b**) Fibrosarcoma – adult: pleomorphic tumor cells with hyperchromatic nuclei of varying shape and rich, distinct cytoplasm. Collagen fragments are often found. MGG



Spindle Cell Sarcomas, Cytological Findings, Fig. 6 Spindle cell GIST: tumor cells with spindle-shaped nuclei and poorly defined cytoplasm. Large tissue fragments are always present. MGG

Spindle cell GIST: spindle tumor cells with oval nuclei and bland chromatin. The cytoplasm is poorly defined. Cell-rich tumor fragments (Fig. 6).

Immunophenotype

Leiomyosarcoma: vimentin, desmin, caldesmon.
Malignant peripheral nerve sheet tumor: vimentin, glial fibrillary acidic protein (GFAP), S100.
Monophasic synovial sarcoma: vimentin, CD99, Bcl-2, S100, EMA (some cells).
Dermatofibrosarcoma protuberans: vimentin, CD34.
Fibrosarcoma – infantile: vimentin, 30% express actin.
Fibrosarcoma – adult: vimentin positivity. Some cases also express actin.
Spindle cell GIST: vimentin, CD34, CD117, and DOG1 positive.

Molecular Features

Leiomyosarcoma: no consistent aberrations.
Malignant peripheral nerve sheet tumor: ?
Monophasic synovial sarcoma: t(X;18).
Dermatofibrosarcoma protuberans: t(17;22).
Fibrosarcoma – infantile: t(12;15). Trisomy for chromosomes 8, 11, 17, and 20.
Fibrosarcoma – adult: no characteristic abnormalities.
Spindle cell GIST: C-kit mutation.

Differential Diagnosis

Leiomyosarcoma: various spindle cell sarcomas, schwannoma.

Malignant peripheral nerve sheet tumor: various spindle cell sarcomas, schwannoma.
Monophasic synovial sarcoma: various spindle cell sarcomas, solitary fibrous tumor.
Dermatofibrosarcoma protuberans: neurofibroma, fibrous histiocytoma, fibrosarcoma.
Fibrosarcoma – infantile: fibrous hamartoma, fibromatosis.
Fibrosarcoma – adult: fibrous histiocytoma, fibromatosis, various spindle cell sarcomas.
Spindle cell GIST: various spindle cell tumors

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Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings

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Synonyms

SCC

Definition

Malignant tumor of surface epithelial cells of the skin or the mucosae.

Clinical Features

- **Incidence**
  10% of all the eyelid malignancies.
- **Age**
  Middle-old age.
- **Sex**
  No gender predilection.
- **Site**
  Lower lid prevalence.
- **Treatment**
  Surgery, chemotherapy, and radiotherapy.
- **Outcome**
  Favorable in early detection and proper treatment.

Macroscopy

Plaque, nodular, and/or ulcerative lesion.

Microscopy

Scraping samples of ▶ squamous cell carcinoma are better observed with Papanicolaou stain in

which squamous cytoplasmic differentiation is more evident. The cytological pattern may vary according to cellular differentiation, which ranges from extremely well differentiated to anaplastic. When almost all cells show indirect signs of parakeratosis, such as wide and extremely keratinizing cytoplasm with retention of small pyknotic nuclei, but the smear lacks cells from a possible lesion beneath, cytological diagnosis should be delayed. A variable number of granulocytes may also be present in the smears, depending on the presence of necrosis. Inflammatory infiltrate may also characterize non-tumoral ulcerated lesions in which scraping may be requested; in this case, the scraping should be effected at the edge of the lesion and not at the center. It may also be useful to remember that benign ulcerated lesions are generally more painful when scraped than tumors, produce fewer cellular smears, and obviously yield cells with a lesser degree of atypia.

Differential Diagnosis

Bowen disease, BCC

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Squamous Cell Carcinoma, Effusions

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Synonyms

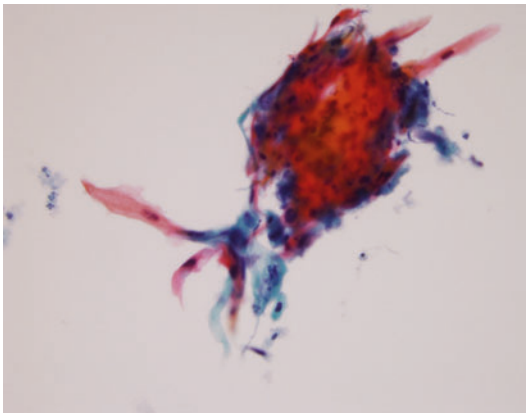
Effusions with metastatic squamous carcinoma

Definition

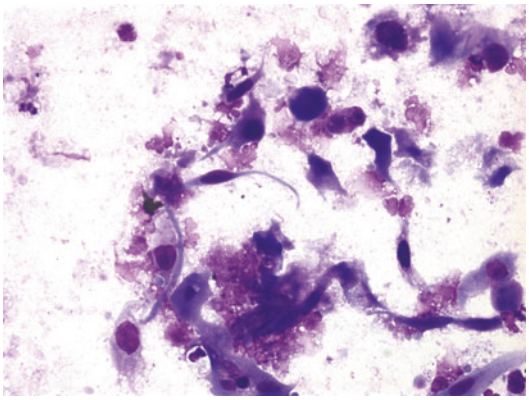
By all accounts, metastatic squamous cell carcinoma to a serous cavity is a rare event (Bibbo and Wilbur 2008; Smith-Purslow et al. 1989). Pleural involvement is most often from tumors of the lung and upper respiratory tract while peritoneal involvement is most often, not-surprisingly, from tumors of the female genital tract (Smith-Purslow et al. 1989; Gamez et al. 2009). Nearly all cases occur in patients with known primary disease.

Microscopy

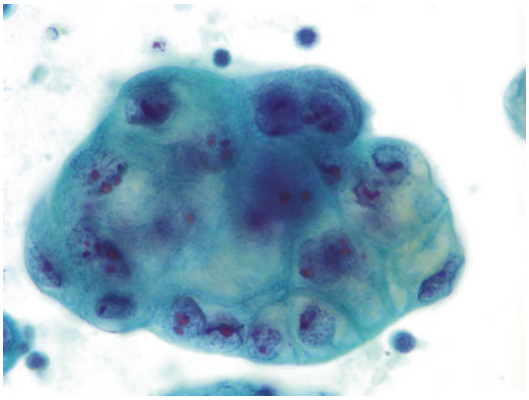
The diagnosis of squamous cell carcinoma is straight-forward when keratin debris and otherwise well-differentiated squamous cells are present. Recognition of orangeophilia in Pap stained preparations is relatively easy. In general, such cells are flat, with angular sharp borders, amphophilic dense cytoplasm, and a hyperchromatic, sometimes smudged nucleus (Figs. 1, 2). They can be seen in flat sheets as well as singly in wildly pleomorphic shapes and sizes (tadpole, fiber, multinucleated giant cells, etc.) Occasionally, keratin pearls can even be appreciated. It is more likely, however,



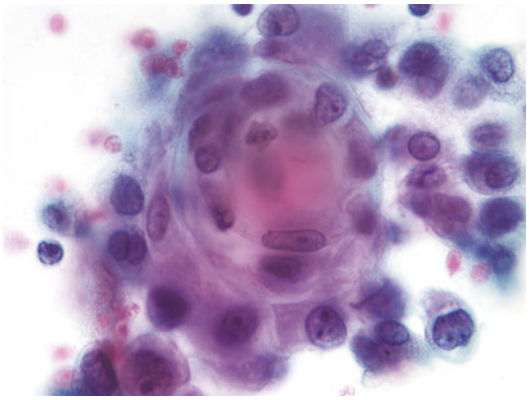
Squamous Cell Carcinoma, Effusions, Fig. 1 Medium power Papanicolaou stain demonstrates atypical keratinized cells from a well-differentiated squamous cell carcinoma in a peritoneal fluid (Pap stain)



Squamous Cell Carcinoma, Effusions, Fig. 2 Medium power Diff-Quik stain demonstrates atypical keratinized cells from the same well-differentiated squamous cell carcinoma (Diff-Quik stain)

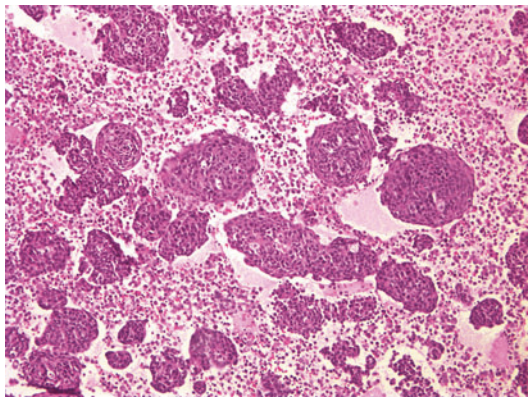


Squamous Cell Carcinoma, Effusions, Fig. 3 High power Papanicolaou stain demonstrates a large cell cluster from a poorly differentiated squamous cell carcinoma. Notice the well-defined cytoplasmic borders and the conspicuous nuclear atypia. Also notice the cytoplasmic glycogen staining in yellow, a finding that is not uncommon (Pap stain)

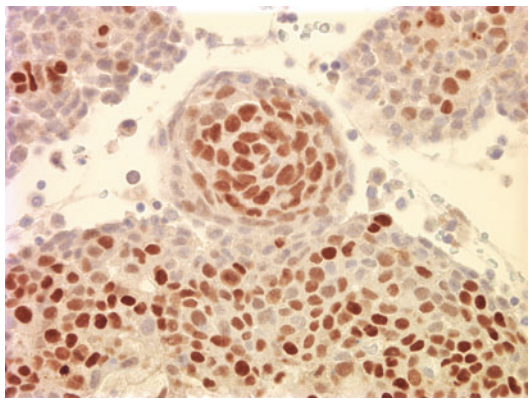


Squamous Cell Carcinoma, Effusions, Fig. 4 High power Papanicolaou stain demonstrates a moderately differentiated squamous cell carcinoma. Notice the onion skin-like wrapping of the cells, recapitulating the squamous eddies seen in histological sections (Pap stain)

that squamous cell carcinoma will be non-keratinizing and poorly differentiated (Smith-Purslow et al. 1989). These cases are characterized by cells with increased nuclear-cytoplasmic ratios, cytoplasmic vacuolization, and enlarged nuclei with coarse chromatin (Fig. 3). These cells are often loosely cohesive and can form three-dimensional aggregates mimicking poorly differentiated adenocarcinoma. Features aiding the differentiation of squamous cell carcinoma from adenocarcinoma include centrally placed nuclei, well-defined cell borders, and the relative dyshesiveness of the cells. Small clusters with cellular wrapping representing attempts to form pearls can be a clue to this diagnosis (Fig. 4). When possible, preparation of cell-block material for ancillary immunohistochemistry can be of great assistance. Cell-blocks are also very useful since the squamous nature of the cells and squamous eddies in the moderately differentiated cases are readily recognized (Fig. 5). Mesothelial proliferations can also be confused with metastatic squamous cell carcinoma. Mesothelial cells are characterized by a foamy skirt and two-tone



Squamous Cell Carcinoma, Effusions, Fig. 5 High power H&E stain of cell-block demonstrates a cellular moderately differentiated squamous cell carcinoma forming large aggregates. Notice the onion skin pattern consistent with squamous eddies (H&E stain)



Squamous Cell Carcinoma, Effusions, Fig. 6 Medium power p63 stain on a cell-block section demonstrating positive nuclei in a poorly differentiated squamous carcinoma

cytoplasm and, most often, will not demonstrate the irregular nuclear contours and coarse hyperchromatic chromatin seen in squamous cell carcinoma. Be aware that anucleate squamous cells can originate from a variety of processes other than metastatic squamous cell carcinoma. In all cases, clinical correlation is essential.

Ancillary Studies

Recognition of intercellular bridging and anucleate keratin debris can be aided by preparation of cell-block material and H&E staining. p63 and p40 (Fig. 6) are useful immunostains expressed in the nuclei of squamous cells that can reliably distinguish metastatic squamous cell carcinoma from both adenocarcinoma and mesothelial proliferations.

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Squamous Cell Carcinoma, In Situ

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Synonyms

Cervical intraepithelial neoplasia 3 (CIN 3); High-grade squamous intraepithelial lesion (HSIL) corresponding to CIN3; High-grade dysplasia; Severe dysplasia; Severe dyskaryosis; Pre-cancer

Definition

Cervical squamous cell in situ carcinoma, designated more commonly as CIN 3, is characterized by major loss of orderly maturation of the epithelium with the deepest two-thirds or more of the epithelium replaced by immature abnormal cells with abnormal nuclei and atypical mitoses (Jenkins 2007). It is the potentially premalignant transformation and abnormal growth (dysplasia) of squamous cells confined to the epithelial layer and its glands (Kumar et al. 2007).

Clinical Features

- **Incidence**
  Each year, over 40,000 women are found to have CIN 2 or CIN 3. Almost all of them are successfully treated. However, over 3,000 new cases of cervical cancer are diagnosed each year in the UK and 11,000 in the USA (Dobbs et al. 2000).
- **Age**
  Younger women are more likely to have CIN 3 than older women. The risk is low during the teens but is highest during the ages of 20–29, slowly decreasing thereafter (Dobbs et al. 2000).
- **Sex**
  It is a disease of females only.
- **Site**
  Cervix uteri.
- **Treatment**
  All women over the age of 20 should have a cervical screening test at least every five years. In England, women between 25 and 64 years of age are screened for cervical neoplasia under NHS (National Health Services) cervical screening programme. Cervical Pap test by conventional or liquid-based cytology method is recommended. Persistent human papilloma virus (HPV) infections are now recognized as the cause of essentially all cervical cancers. Women with a cytologic result of severe dyskaryosis (HSIL) should undergo colposcopy with biopsy confirmation and endocervical assessment. Treatment methods commonly used to treat cervical lesions include cryosurgery (freezing that destroys tissue), LEEP (loop electrosurgical excision procedure/LLETZ (large loop excision of the transformation zone), or the removal of tissue using a hot wire loop), and conization (surgery to remove a cone-shaped piece of tissue from the cervix and cervical canal) (http://www.cancer.gov/cancertopics/factsheet/Risk/HPV).
- **Outcome**
  Women with CIN 3 and incomplete excisional margins are the most likely to have recurrent or residual disease (Dobbs et al. 2000). With CIN 3, the likelihood of regression is only 33% and of progression 60–74% (in various studies) (Kumar et al. 2007). **HPV triage and test of cure** (NHS cervical screening programme, UK) – Women whose cytology test shows borderline change or mild dyskaryosis can have an HPV test. If this test is positive for high-risk HPV, they are referred to colposcopy. If negative, they return to routine 3- or 5-year recall, depending on age. All women who have been treated for CIN have an HPV test and cytology test at six months following treatment. Women who are HPV-negative with normal cytology proceed to a 3-year recall period. Women who are either high-risk HPV-positive or have abnormal cytology are referred back to colposcopy.
- **Colposcopic Diagnosis**
  It depends on the recognition of intensity of aceto-whitening, margins, and surface contour of acetowhite areas, vascular features, and color changes after iodine application.

  High-grade CIN is associated with thick, dense, dull, opaque, or grayish-white acetowhite areas with well-demarcated, regular margins, which may be raised and rolled out (http://screening.iarc.fr/doc/colpochapter07.pdf). The surface contour of the acetowhite areas tends to be less smooth, or irregular and nodular (http://screening.iarc.fr/doc/colpochapter07.pdf). Vascular changes with coarse punctation and/or coarse mosaics in acetowhite areas occur in high-grade lesions (http://screening.iarc.fr/doc/colpochapter07.pdf).

Macroscopy

Cone and loop biopsies are performed for pre-invasive lesions. Abnormality may not be appreciable on the gross inspection of the tissue biopsy. These are serially sliced at 2–3-mm intervals (Scurry et al. 1993; Rosai 2004), from one edge to the other in a sagittal and parasagittal plane perpendicular to the transverse axis of the external os.

Microscopy

Cervical Cytology

Severe dyskaryosis (HSIL) is characterized by (Titmuss and Adams 2007):

- Sheets and syncytial groups (Fig. 1)
- A tendency to have more clusters and single dyskaryotic/ dysplastic cells
- Nuclei with an abnormal three-dimensional asymmetry
- High N:C ratio, with the nuclei occupying two-thirds or more of the cell's diameter with scant cytoplasm
- Nuclear pleomorphism and hyperchromasia (Fig. 2)
- Irregular coarse chromatin pattern
- Nuclear membrane irregularities
- Prominent nucleoli may be seen
- Few bizarre shaped cells

Histopathology

Three histological subtypes (Titmuss and Adams 2007) of CIN 3:

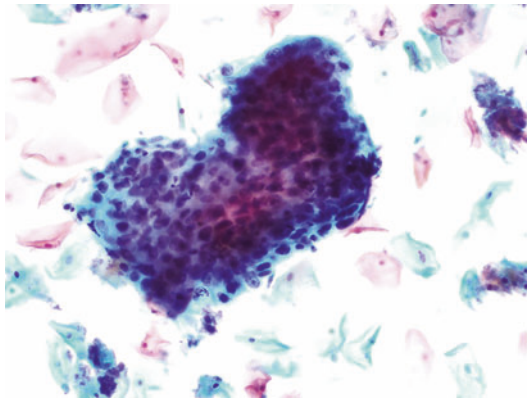
1. Small-cell non-keratinizing
2. Large-cell non-keratinizing
3. Keratinizing CIN3.

In cone/loop biopsies, the status of ectocervical, endocervical, and deep lateral/radial resection margins should be commented along with their involvement by CIN, cervical glandular intraepithelial lesion (CGIN), or invasive carcinoma.

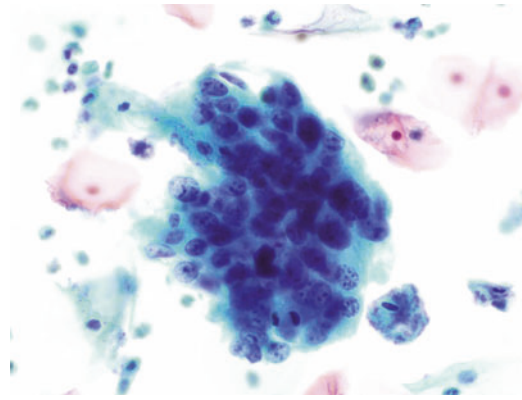
Differential Diagnosis of Severe Dyskaryosis (HSIL) in Cytology Smears

1. Severe inflammatory or reactive changes
2. Radiation and chemotherapy changes
3. Invasive squamous cell carcinoma or adenocarcinoma
4. Herpes simplex virus

Small-cell severe dyskaryosis (HSIL) in smears can be difficult to distinguish from lymphocytes, histiocytes, endometrial cells, and immature metaplastic cells. Pale cell dyskaryosis is a particular problem when associated with small-cell severe dyskaryosis (HSIL). Keratinizing severe dyskaryosis (HSIL) in smears should be



Squamous Cell Carcinoma, In Situ, Fig. 1 A syncytial cluster of dyskaryotic cells showing loss of polarity (Pap ×40X)



Squamous Cell Carcinoma, In Situ, Fig. 2 The individual cells show nuclear pleomorphism, high nuclear-cytoplasmic ratio, irregular coarse chromatin, and occasional conspicuous nucleoli. Occasional mitotic figure is seen (Pap ×100X)

differentiated from small keratinized cells in atrophic smears (Titmuss and Adams 2007).

Immunophenotyping and Molecular Features

HPV detection techniques include immunocytochemical techniques, dot blot, southern blot, filter in situ hybridization, Hybrid Capture 2 (HC2) assay (Digene, MD), PCR techniques, and genotyping assays (Gupta et al. 2010). Commonly used HPV DNA detection techniques include Hybrid capture 2 (HC2), in situ hybridization, and polymerase chain reaction (Castle et al. 2007).

There are commercially available immunocytochemical assays for the simultaneous qualitative detection of the p16INK4a and Ki-67 proteins in cervical cytology preparations. Preliminary studies have shown that the performance of ProEx C detection of CIN 2/3 in cervical LBC slides has a sensitivity of 85.3% and specificity of 71.7% (Shi et al. 2007).

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Squamous Cell Carcinoma, Invasive

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Synonyms

Invasive squamous cell cancer of cervix

Definition

Invasive tumor or malignant tumor is characterized by uncontrolled cell division and invasion through the basement membrane into the underlying stroma. The most common cervical carcinomas are squamous cell carcinomas (SCC) (75%), followed by adenocarcinomas and adenosquamous carcinomas (20%), and small-

cell neuroendocrine carcinomas (<5%) (Kumar et al. 2007).

Clinical Features

- **Incidence**
  Invasive cervical cancer accounts for 2.5% of all cancers that afflict women in the USA (http://www.healthscout.com/ency/416/115/main.html). About 13,500 cases of invasive carcinoma of the cervix are diagnosed in the USA each year (http://www.healthscout.com/ency/416/115/main.html). In 2007, 2,828 new cases of cervical cancer were diagnosed in the UK, making it the eleventh most common cancer in females and accounting for around 2% of all female cancers (http://info.cancerresearchuk.org/cancerstats/types/cervix/incidence).
- **Age**
  Cervical cancer is the most common cancer in females under 35, with 702 new cases diagnosed in the UK in 2007 (http://info.cancerresearchuk.org/cancerstats/types/cervix/incidence). The incidence rate for cervical cancer is highest for those aged 30–39, reaching around 17 per 100,000 women. Although rates decrease for the following age-groups, another peak is reached in women over 70 (http://info.cancerresearchuk.org/cancerstats/types/cervix/incidence).
- **Sex**
  It is a disease of females only.
- **Site**
  Cervix Uteri
- **Symptoms**
  They may include vaginal bleeding, unusual vaginal discharge, pelvic pain, and/or post-coital bleeding.
- **Causes and Risk Factors**
  Major risk factors include initiation of sexual activity at an early age, multiple sexual partners, infection with human papilloma virus 16 and 18, and smoking. Ninety to ninety-five percent of SCC of the cervix contains the human papilloma virus (HPV) DNA.
- **Cervical Screening**
  Screening is done by cervical cytology/Pap smear test. Variable policies for the age group to be screened and treatment of the screening exist throughout the world. In England, women from age 25 to 64 are invited for cervical screening (http://info.cancerresearchuk.org/spotcancerearly/screening/cervicalcancerscreening/). Women aged 25–49 are invited every 3 years. After that, women are invited every 5 years (http://info.cancerresearchuk.org/spotcancerearly/screening/cervicalcancerscreening/). HPV Hybrid capture test for high-risk HPV is also performed in samples with atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL). Primary screening with HPV test instead of Pap test is also being explored in different countries.
- **The FIGO (International Federation of Gynaecology and Obstetrics) Staging of Cervical Cancer**
  Stage I disease is localized to the cervix while stage II tumors involve adjacent upper vagina or invade the parametrium lateral to the cervix. In stage III, the tumor extends to involve the distal vagina or the pelvic sidewall on one or both sides and/or causes ureteral obstruction. Stage IV patients have distant metastasis or invasion of the bladder or rectum (Jones 2006).

  In the UK, microinvasive carcinoma is considered to be synonymous with FIGO stage IA1 and IA2 disease in most, but not all, institutions. In the USA, the term is synonymous with stage IA1 disease (Shepherd 1995).
- **Treatment**
  The usual surgical procedure for cervical carcinoma is a radical hysterectomy and lymph node dissection for stage 1 and 2A tumors. Advanced tumors are treated with radiation therapy and/or chemotherapy. Early tumors may be treated by LEEP (loop electrosurgical excision procedure/LLETZ (Large loop excision of the transformation zone) excision/cone biopsy/trachelectomy, especially if fertility is to be preserved.

- **Outcome**
  Five-year survivals is as follows: Stage 0 (pre-invasive), 100%; stage 1, 90%; stage 2, 82%; stage 3, 35%; and stage 4, 10% (Kumar et al. 2007).

Macroscopy

SCC of the cervix develops in the region of the transformation zone and ranges from microscopic foci of early stromal invasion to grossly identifiable tumors encircling the os (Kumar et al. 2007). The tumors can be invisible or exophytic. Tumors may encircle the cervix and penetrate into the underlying stroma, producing a "barrel cervix."

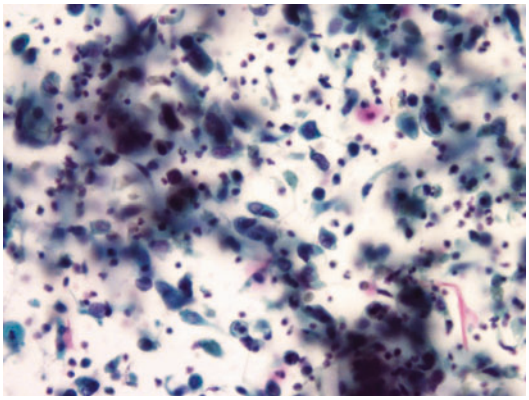
Microscopy

Histologically, it is divided into well-differentiated (keratinizing), moderately differentiated (large-cell), and poorly differentiated (small-cell) SCC (Titmuss and Adams 2007).

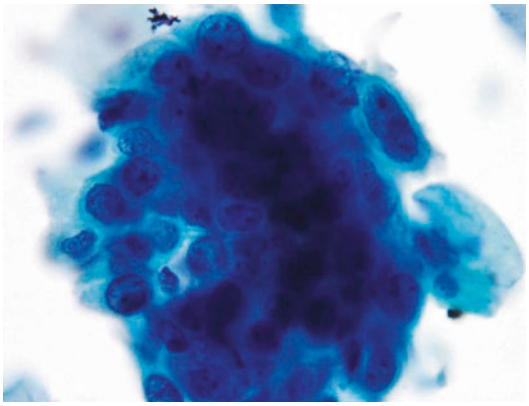
Cytomorphology

Well-differentiated keratinizing SCC – It is characterized by highly pleomorphic, irregular, keratinized, orangeophilic single cells and sheets, with irregular and hyperchromatic nuclei, very dense homogeneous chromatin, and enlarged nucleoli. Bizarre cells, tadpole cells, and fiber cells may be seen with dirty background, indicating tumor diathesis (Fig. 1) (Titmuss and Adams 2007).

Moderately differentiated (large-cell) SCC – It is the most common type of SCC. It is characterized by groups and sheets of malignant cells as well as dispersed population of cells. Nuclei occupy at least half the cell's diameter with considerable nuclear pleomorphism. The chromatin is coarsely granular, irregularly distributed, with prominent windowing. Large irregular nucleoli are frequently seen. The cells have well-defined cellular outline with dense basophilic cytoplasm (Fig. 2) (Titmuss and Adams 2007).



Squamous Cell Carcinoma, Invasive, Fig. 1 Smear showing bizarre cells and tadpole cells with dense orangeophilic cytoplasm in the case of keratinizing SCC (PAP ×100X)



Squamous Cell Carcinoma, Invasive, Fig. 2 A cluster of malignant cells showing nuclear pleomorphism, coarsely granular chromatin, conspicuous nucleoli, and dense basophilic cytoplasm (Pap×100X)

Poorly differentiated (small-cell) SCC – It is characterized by many small, poorly differentiated epithelial cells, which may be present in syncytial groups or tight clusters. The cells have high nuclear: cytoplasmic ratio, hyperchromatic, pleomorphic nuclei, stippled chromatin, and scanty cytoplasm (Titmuss and Adams 2007).

Immunophenotype and Molecular features

Functional biomarkers for cervical precancer and cancer include cell cycle markers such as

p16INK4a, Ki-67, p53, retinoblastoma protein (pRb), p21, p27, MCM5, CDC6, cyclin A, E, and D, markers of squamous differentiation cytokeratin (CK) such as CK14 and CK13, and other molecules such as involucrin, telomerase, survivin, VEGF, FHIT, and several others (Gupta et al. 2010). p16INK4a is overexpressed in cervical dysplasia/cancer (Murphy et al. 2005; Dehn et al. 2007). The sensitivity and specificity of p16 for high-grade lesions has been reported to be 77% and 57%, respectively (Benevolo et al. 2008). ProEx C is a cocktail of monoclonal antibodies against proteins associated with aberrant S phase cell cycle induction (topoisomerase IIA, minichromosome maintenance protein 2) (Boucher et al. 2007). The increased proliferation of cervical epithelial cells caused by deregulated HPV oncogene expression is reflected in the activation of proliferation markers such as Ki-67 (MIB-1).

Fluorescence *In Situ* Hybridization (FISH) can be performed on cervicovaginal liquid-based preparations to detect gain of 3q26. Gain of 3q26 is associated with HSIL (high-grade squamous intraepithelial lesion) and SCC (squamous cell carcinoma) (Gupta et al. 2010).

Differential diagnosis in cytologic smears includes squamous intraepithelial lesions (SIL), repair and regenerative changes, sarcoma, and endocervical/endometrioid adenocarcinoma (Titmuss and Adams 2007).

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Squamous Intraepithelial Lesion, High Grade (HGSIL)

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Synonyms

Cervical intraepithelial neoplasia grade 2 and 3; Moderate and severe dyskaryosis; Moderate and severe dysplasia

Definition

The term squamous intraepithelial lesion (SIL) describes a group of cervical proliferations that may, if untreated, progress to invasive squamous cell carcinoma of the cervix. Historically, these lesions were thought to represent a spectrum of biologically interrelated intraepithelial change. More recently, epidemiological research suggests that SIL is more appropriately classified into two groups: low-grade lesions (low-grade squamous intraepithelial lesion, or LSIL), which are largely transient and highly likely to regress without

clinical intervention, and a separate group of high-grade lesions (high-grade squamous intraepithelial lesion, or HSIL) having a greater propensity for malignant transformation. The SIL nomenclature is encompassed within the Bethesda System (Solomon et al. 2002) for cytological diagnosis but is also conveniently used for reporting cervical histology. Both LSIL and HSIL are caused by infection with one or more types of ▶ human papillomavirus.

The existence of several different classification systems describing cervical intraepithelial lesions is the source of much confusion among pathologists and gynecologists worldwide. HSIL includes lesions also called moderate and severe dyskaryosis (Cancer Screening Programmes 2013), moderate and severe dysplasia, and cervical intraepithelial neoplasia (CIN) grades 2 and 3 (Richart 1968).

Clinical Features

- **Incidence**
  As a symptomless condition, HSIL is generally only detected through cervical screening. The best estimates of HSIL rates are therefore derived from population-based screening programs. In the United Kingdom, for instance, there were 34,045 cytology samples reported as moderate and severe dyskaryosis in 2009–2010, comprising 1.1% of adequate samples (The NHS Information Centre and Population Statistics Team 2010). High-grade intraepithelial neoplasia or invasive cancer was confirmed in over 80% of cases. In a survey of over 1,000 laboratories in the United States in 2003, the median reporting rate for HSIL was 0.5% (Davey et al. 2004).
- **Age**
  Most cases of HSIL are registered in women under 45, with peak incidence in the 25–29 age group.
- **Sex**
  SIL terminology is relevant only in females.
- **Site**
  SIL terminology is used only for the reporting of cervical and vaginal samples.
- **Treatment**
  Because of the relatively high rates of progression from HSIL to invasive cancer, histologically confirmed HSIL is usually treated. When HSIL is diagnosed during pregnancy, treatment is generally delayed until 2–3 months postpartum. Excisional or ablative techniques are most commonly used and are equally effective.

  Loop electrosurgical excision procedure (LEEP), also known as large loop excision of the transformation zone (LLETZ), is a straightforward technique that makes use of an electrified wire loop to cut through and excise the whole transformation zone while at the same time achieving hemostasis. Complete excision may require one or multiple passes of the loop, depending on the size of the transformation zone and extent of the lesion.

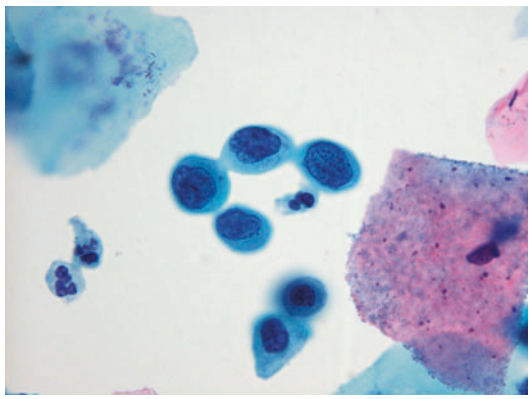
  Ablative methods of treatment include cryotherapy, electrocoagulation, cold coagulation, and laser ablation. Cryotherapy is the most cost effective of the ablative methods and makes use of a cryoprobe and compressed refrigerant gases to freeze and destroy an area of cervical tissue. If good contact is maintained between the probe tip and the cervix, temperatures below −70 °C can be achieved, which is sufficient to induce tissue necrosis and cell death. Although relatively inexpensive, cryotherapy has two main disadvantages: it is suitable only for the treatment of lesions that are located entirely on the ectocervix and the procedure does not yield tissue for histological assessment.
- **Outcome**
  HSIL is the true precursor of squamous cell carcinoma of the cervix. Compared with LSIL, available data suggest that at least 12% of high-grade lesions will progress to invasive cancer if left untreated (Ostor 1993). The transition time from the precancerous stages to invasive cervical cancer is quite variable but is generally long, ranging from 10 to 20 years. Noteworthy is the observation that a substantial proportion of high-grade precursor lesions, perhaps as many as 30%, will regress to normal if left untreated.

Macroscopy

HSIL is recognized colposcopically by the intensity of epithelial whitening following the application of 5% acetic acid solution, the nature of the margins and surface contour of the acetowhite area, alterations in the pattern of surface capillaries, and color changes after the application of Lugol's iodine. HSIL is often seen as well-demarcated, densely acetowhite areas with coarse punctation or mosaicism. High-grade lesions are generally more extensive and complex than low-grade lesions. Severe lesions may have raised, rolled, or peeling margins and do not stain upon application of Lugol's iodine.

Microscopy

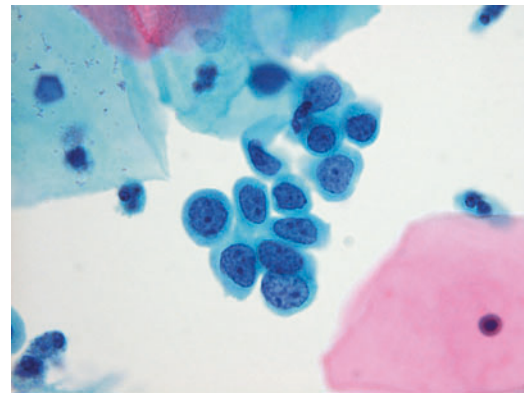
Cytologically, HSIL is characterized by the presence of immature basaloid cells with a high nucleocytoplasmic ratio and containing enlarged, hyperchromatic, and coarsely granular nuclei. The nuclear border is often highly irregular. Nucleoli are rarely seen and the cytoplasm is most often densely cyanophilic. The cytopathic effect of human papillomavirus infection is usually absent. Although HSIL cells are often found isolated or in small clusters (Figs. 1 and 2), a common cytological presentation of HSIL is the hyperchromatic crowded cell group, which is typically a large, irregularly shaped, three-dimensional group of cells with densely crowded and darkly stained nuclei (Fig. 3).



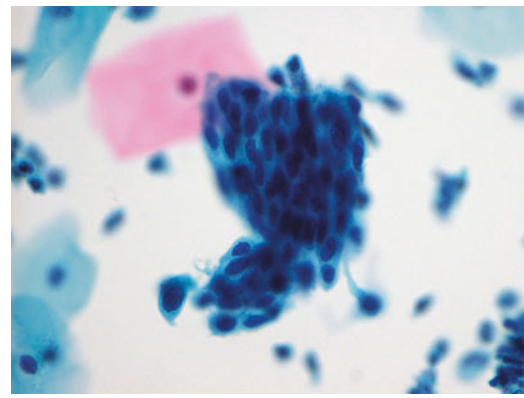
Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 1 Isolated HSIL cells (Pap stain)

Immunophenotype

Immunophenotyping studies of SIL have demonstrated a plethora of putative diagnostic, prognostic, and predictive biomarkers. Most research has focussed on expression markers associated with progressive HPV infection. In this respect, proteins associated with inappropriate activation of the cell cycle have been at the forefront of biomarker research. Examples include the overexpression of MCM (minichromosome maintenance) and CDC (cell division cycle) proteins, $p16^{INK4a}$, PCNA (proliferating cell nuclear antigen), Ki-67 (MIB-1), and various growth factors and their receptors. Underexpressed proteins with



Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 2 A loose cluster of HSIL cells (Pap stain)

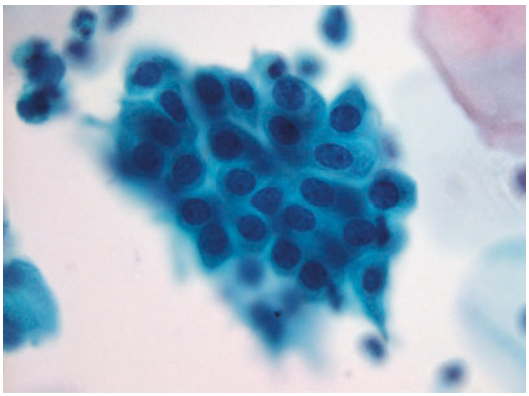


Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 3 A hyperchromatic crowded group of HSIL cells (Pap stain)

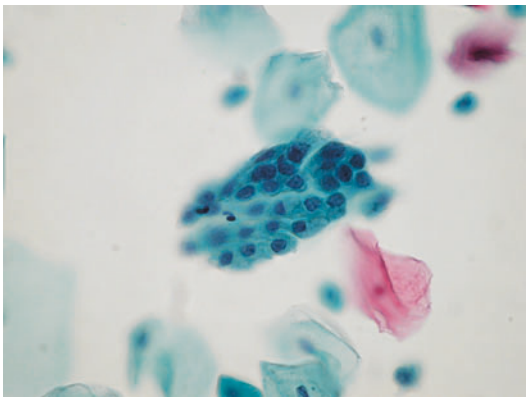
potential application as biomarkers in cervical pathology include the p53 and pRb tumor suppressor proteins.

Molecular Features

HSIL is the result of persistent infection with high-risk subtypes of HPV, particularly types 16 and 18. In low-grade lesions viral DNA is found exclusively in an unintegrated form, i.e., it exists freely within the nucleus of host cells. In high-grade lesions however, the nucleic acid of the virus is integrated into the host cell genome in a high proportion of cases. The site of viral integration is not uniform across lesions. Integration results in the disruption of the E2 gene of the viral genome and deregulation of the E6 and E7 transforming genes. At this stage of the life cycle of HPV, abnormal cellular proliferation characteristic of HSIL can be seen both cytologically and histologically. In contrast to LSIL, a substantial proportion of high-grade lesions display extreme chromosomal aberrations and aneuploidy. Epigenetic modifications, such as hypo- and hypermethylation, are also frequently observed. Highly overexpressed genes in progressive HSIL lesions include CDK (cyclin-dependent kinase) inhibitor 2A, prostaglandin E synthase, MCM, cyclin B1, TOP2alpha, and E2F transcription factor 1.



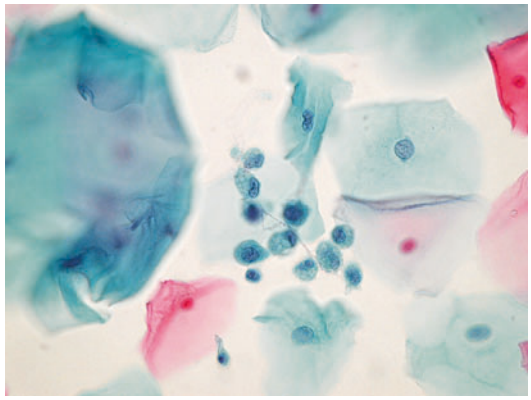
Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 4 Immature squamous metaplastic cells. Note finely granular chromatin and smooth nuclear borders (Pap stain)



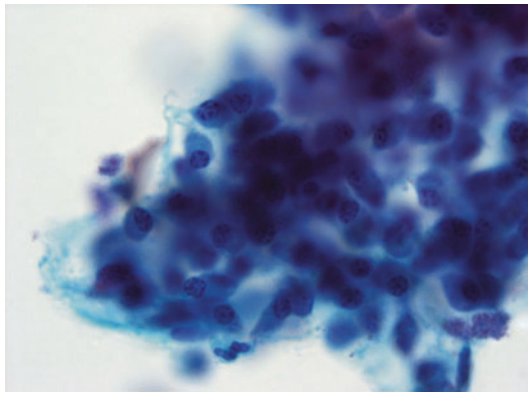
Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 5 Degenerative changes in endocervical cells (Pap stain)

Differential Diagnosis

There are several interpretative pitfalls to be aware of when considering a diagnosis of HSIL in a cytology sample. The cells of immature squamous metaplasia have relatively large nuclei that may be hyperchromatic (Fig. 4). If the uniformity of the nuclear chromatin is not recognized, then misdiagnosis of HSIL is a real possibility. Reserve cell hyperplasia and normal endocervical cells are less likely to be confused with HSIL, but care is required when these cells show degenerative features or when the cytoplasm of such cells is denuded (Fig. 5). Histiocytes, plasma cells, and endometrial cells are small cells with a high nucleocytoplasmic ratio and can closely resemble HSIL. The clue to their correct recognition lies mainly in the interpretation of nuclear chromatin, in nuclear membranes, and in the exfoliation pattern of these cells. Histiocytes usually appear as single cells or very loose clusters. Nuclei are characteristically reniform and finely granular (Fig. 6). Plasma cells are a rare finding in cervical cytology and are generally only seen in large numbers in the condition follicular cervicitis. The nuclei of plasma cells usually have a striking "clockface" configuration, readily distinguishing them from HSIL (Fig. 7). The most characteristic feature of endometrial cells that in most cases permits their

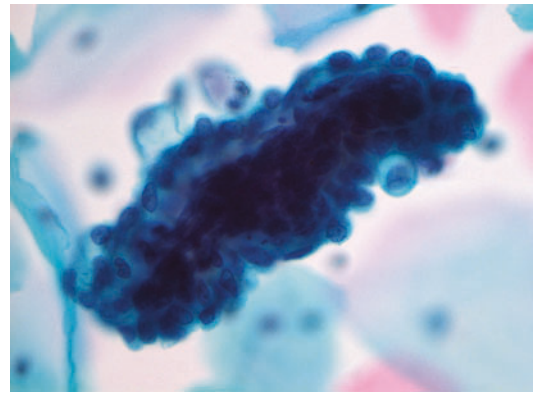


Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 6 Histiocytes. Note foamy cytoplasm and reniform nuclei (Pap stain)



Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 7 Plasma cells. Note "clockface" chromatin pattern (Pap stain)

easy identification is the pattern of exfoliation. Endometrial cells are commonly seen as a tightly cohesive plaque of cells and sometimes as a "wreath" or biphasic group (Fig. 8). In the presence of an intrauterine contraceptive device, reactive changes in endometrial cells exhibit enlarged rounded nuclei with prominent nucleoli and prominent cytoplasmic vacuolation. It is important to distinguish these changes from those of HSIL.



Squamous Intraepithelial Lesion, High Grade (HGSIL), Fig. 8 Endometrial cells (Pap stain)

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Squamous Intraepithelial Lesion, Low Grade (LGSIL)

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Synonyms

Cervical intraepithelial neoplasia grade 1; Mild dyskaryosis; Mild dysplasia

Definition

The term squamous intraepithelial lesion (SIL) describes a group of cervical proliferations that may, if untreated, progress to invasive squamous cell carcinoma of the cervix. Historically, these lesions were thought to represent a spectrum of biologically interrelated intraepithelial change. More recently, epidemiological research suggests that SIL is more appropriately classified into two groups: low-grade lesions (low-grade squamous intraepithelial lesion, or LSIL), which are largely transient and highly likely to regress without clinical intervention, and a separate group of high-grade lesions (high-grade squamous intraepithelial lesion, or HSIL) having a greater propensity for malignant transformation. The SIL nomenclature is encompassed within the Bethesda System (Solomon et al. 2002) for cytological diagnosis but is also conveniently used for reporting cervical histology. Both LSIL and HSIL are caused by infection with one or more types of ▶ human papillomavirus (HPV).

The existence of several different classification systems describing cervical intraepithelial lesions is the source of much confusion among pathologists and gynecologists worldwide. LSIL includes lesions also called mild dyskaryosis (dyskaryosis is the terminology recommended by the National Health Service Cervical Screening Programme (2013)), mild dysplasia (the term dysplasia is favored by the World Health Organization), and cervical intraepithelial neoplasia (CIN) grade 1 (the CIN concept was introduced by Richart in the late 1960s (Richart 1968)). Unfortunately, the discovery that HPV is associated with LSIL led to the introduction of several unhelpful terms to describe what is essentially a reversible viral infection. Examples of such terms include koilocytotic atypia, condylomatous atypia, warty atypia, and atypical condyloma.

Clinical Features

- **Incidence**
  The true incidence of LSIL is unknown because the condition is symptomless and largely transient. The problem is compounded by the lack of comparability between different classification systems for low-grade cervical lesions. Apparent prevalence is also dependent on the extent to which a population is screened. In the UK, which has a well-screened population, the reporting rate for mild dyskaryosis (broadly equivalent to LSIL) in 2009–2010 was 1.9% of adequate samples (The NHS Information Centre and Public Health Indicators and Population Statistics Team 2010). In a survey of over 1,000 laboratories in the USA in 2003, the median reporting rate for LSIL was 2.1% (Davey et al. 2004).
- **Age**
  LSIL is most prevalent in young women (under 30 years) and decreases with age.
- **Sex**
  SIL terminology is relevant only in females.
- **Site**
  SIL terminology is used for the reporting lower anogenital tract samples.
- **Treatment**
  The decision whether to treat LSIL must represent a balance between the need to prevent cervical cancer and the desire to prevent overtreating lesions that are destined to regress. In the vast majority of cases, women diagnosed with LSIL undergo cytological surveillance, with treatment only being offered if lesions persist beyond 18–24 months or progress to HSIL.
- **Outcome**
  The results of a pooled analysis of studies between 1950 and 1993 suggest that around 60% of LSIL will regress to normal over time, 30% will persist as low-grade lesions, 10% will progress to HSIL, and less than 1% would be expected to progress to invasive cancer if left untreated (Ostor 1993).

Macroscopy

Colposcopically, LSIL is recognized by the intensity of epithelial whitening following the application of 5% acetic acid solution, the nature of the margins and surface contour of the acetowhite area, alterations in the pattern of surface

capillaries, and color changes after the application of Lugol's iodine. LSIL is often seen as a well-demarcated, smooth, faintly acetowhite area with an irregular or "feathery" boundary. The surface contour is usually smooth but may have fine mosaicism or punctation. Vascular changes are minimal with equal intercapillary distances. Uptake of iodine is minimal or absent due to a lack of glycogen in intraepithelial lesions.

Microscopy

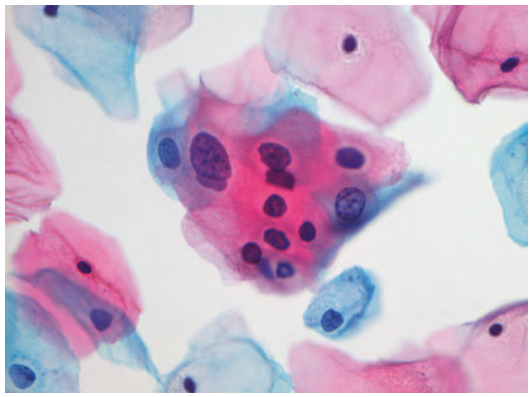
Cytologically, LSIL presents as mature squamous epithelial cells with disproportionate nuclear enlargement (more than 3 times the area of normal intermediate nuclei) but relatively low nucleocytoplasmic ratio (i.e., less than 0.5), irregular nuclear boundaries, and nuclei with granular hyperchromatic chromatin (Fig. 1). Nucleoli are absent or inconspicuous. Superimposed on these changes may be the cytopathic effects of HPV infection, which include koilocytosis (squamous epithelial cells with well-demarcated deep perinuclear vacuoles), cytoplasmic keratinization, and bi- or multinucleation (Fig. 2).

Immunophenotype

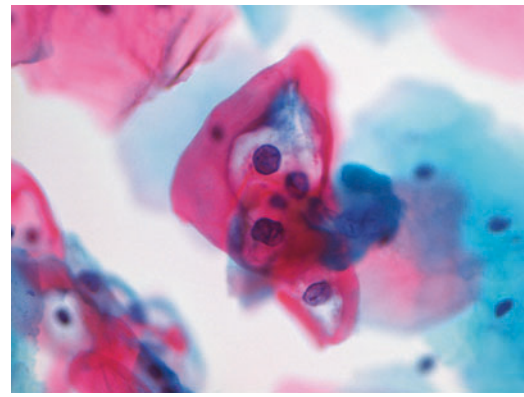
Immunophenotyping studies of SIL have demonstrated a plethora of putative diagnostic, prognostic, and predictive biomarkers. The goal is to identify one or more expression markers that will permit the reliable distinction between harmless lesions destined to regress from those with true malignant potential. Perhaps unsurprisingly, attention has focussed on protein markers associated with progressive HPV infection. In this respect, proteins associated with inappropriate activation of the cell cycle have been at the forefront of biomarker research. Examples include the overexpression of MCM (minichromosome maintenance) and CDC (cell division cycle) proteins, $p16^{INK4a}$, PCNA (proliferating cell nuclear antigen), Ki-67 (MIB-1), and various growth factors and their receptors. Underexpressed proteins with potential application as biomarkers in cervical pathology include the p53 and pRb tumor suppressor proteins.

Molecular Features

Changes at the level of DNA and RNA in SIL and invasive cervical cancer have been well researched and revolve around the life cycle of HPV. It is now known that LSIL results from a productive infection of the basal layer of cervical squamous epithelium, during which there is active viral replication and signs of early histological change. Most of these productive infections resolve spontaneously, but the small number that persists or progresses is molecularly characterized by the expression of the early viral genes E6 and E7. These viral genes



Squamous Intraepithelial Lesion, Low Grade (LGSIL), Fig. 1 LSIL (Pap stain)

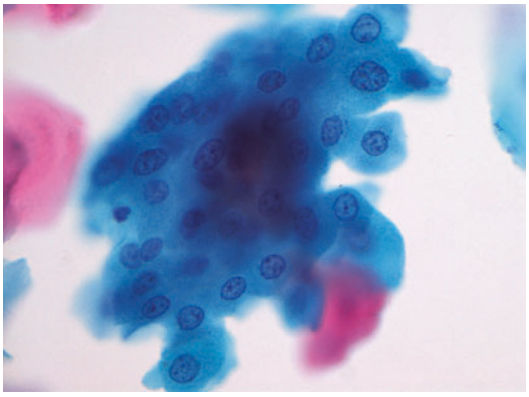


Squamous Intraepithelial Lesion, Low Grade (LGSIL), Fig. 2 LSIL with superimposed HPV changes (Pap stain)

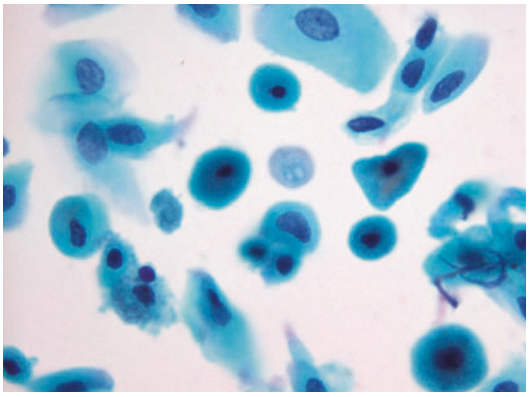
interfere with host cells in a way that may ultimately cause structural and numerical chromosomal alterations. However, flow cytometric and image cytometric studies have demonstrated that the vast majority of low-grade lesions retain chromosomal diploidy. Despite retaining a near-normal chromosome complement, structural alterations in the region of chromosome 3q in SIL are common. Upstream of chromosomal aberrations are changes in several proliferation-associated genes, and some of these have been associated with neoplastic progression of low-grade lesions. The potential for discovering new molecular markers of neoplastic progression is enormous: over 240 genes are known to be upregulated in invasive cervical cancer and a similar number of genes exhibit downregulation (Santin et al. 2005). Among the most highly overexpressed genes are CDK (cyclin-dependent kinase) inhibitor 2A, prostaglandin E synthase, MCM, cyclin B1, TOP2alpha, and E2F transcription factor 1. More recently, epigenetic modifications such as DNA methylation have been noted as early molecular events in the neoplastic progression of low-grade cervical lesions. Only time will tell whether any of these molecules will have clinical utility.

Differential Diagnosis

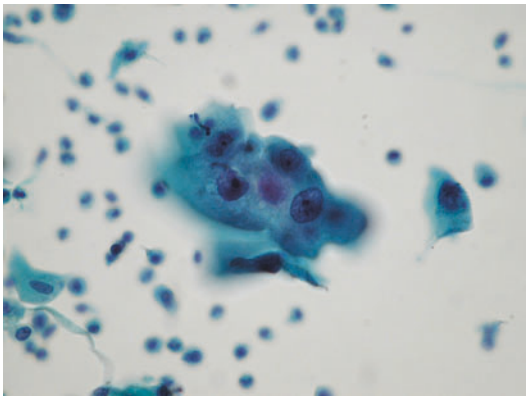
Cytologically, LSIL has morphological similarities to several benign and neoplastic entities, from which it should be distinguished. Cells with reactive cellular changes may display nuclear enlargement and hyperchromasia resembling the changes associated with LSIL (Fig. 3). Careful attention to chromatin pattern, which is characteristically irregular in LSIL and finely granular or pyknotic in reactive cellular changes, should help to prevent misinterpretation. Cell degeneration can result in shrinkage and wrinkling of nuclei, which can resemble the membrane irregularities of LSIL (Fig. 4). The degenerative changes associated with chemotherapy, radiotherapy, and atrophy can be particularly problematic in this respect (Fig. 5). Navicular cells are glycogenated squamous epithelial cells associated with progesterone stimulation and are therefore prominent during



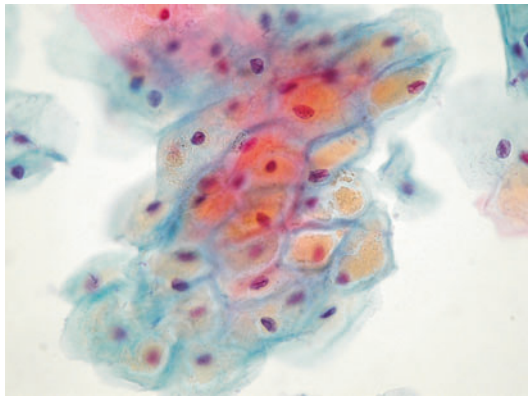
Squamous Intraepithelial Lesion, Low Grade (LGSIL), Fig. 3 Reactive cellular changes resembling LSIL (Pap stain)



Squamous Intraepithelial Lesion, Low Grade (LGSIL), Fig. 4 Cell degeneration mimicking LSIL (Pap stain)



Squamous Intraepithelial Lesion, Low Grade (LGSIL), Fig. 5 Radiotherapy changes (Pap stain)



Squamous Intraepithelial Lesion, Low Grade (LGSIL), Fig. 6 Navicular cells can resemble koilocytosis (Pap stain)

pregnancy and the secretory phase of the menstrual cycle. The accumulation of faintly stained glycogen within the cytoplasm of a navicular cell can give it a similar appearance to a koilocyte (Fig. 6). However, navicular cells rarely have enlarged or hyperchromatic nuclei and the distinction from koilocytosis is straightforward in the vast majority of cases. Verrucous carcinoma is an important, but thankfully rare diagnosis that must be distinguished from LSIL. The cells of this neoplasm are typically hyperkeratotic with hyperchromatic and pyknotic nuclei that belie their malignant nature. Anucleate fragments of highly keratinized cytoplasm may also be a feature. The similarity to low-grade keratinizing lesions can be striking and must rely on careful attention to the nuclear detail in the less keratinized areas of the sample.

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Squamous Metaplasia, Cytological Findings

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Synonyms

Indirect metaplasia

Description

Physiology

In the cervix, squamous metaplasia is a normal dynamic process by which endocervical columnar epithelium is replaced by squamous epithelium in response to a stimulus. Initiation of the process depends upon the position of the squamocolumnar junction (SCJ), a histologically visible abrupt transition between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. Before puberty, the SCJ lies within the endocervical canal, and the delicate endocervical columnar epithelium is relatively well protected from the harsh acidic environment of the vagina. At the onset of puberty and periodically thereafter, the cervix increases in size in response to the secretion of ovarian sex hormones. The increased bulk of the cervix causes the SCJ to evert, thus exposing the endocervical

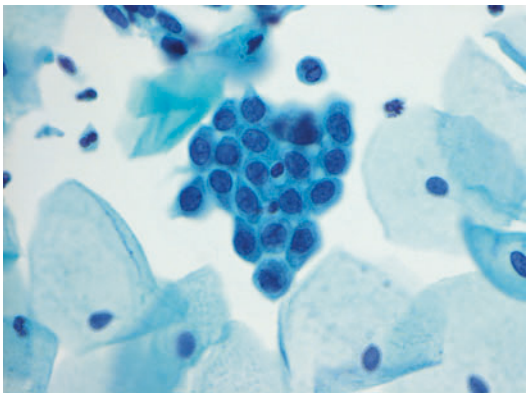
columnar epithelium to the low vaginal pH. The area of exposed endocervical epithelium is known as an ectropion or ectopy. The acidity is thought to act as the stimulus for subcolumnar reserve cells to divide and proliferate, forming an area of reserve cell hyperplasia. Subsequent differentiation of reserve cells into immature squamous metaplasia followed by mature squamous metaplasia completes the process. The area of metaplasia is often referred to as the transformation zone, referring as it does to the zone of transformation from one cell type to another. The process culminates with protective stratified squamous epithelium occupying the transformation zone, thus forming a "new" SCJ within the endocervical canal. The process of squamous metaplasia is a patchy one, often occurring at several different areas in the ectopic epithelium at different times. The transformation zone is peculiarly susceptible to infection by human papillomavirus and neoplastic change, thus making it the prime target for sampling in cervical screening programs.

Histology

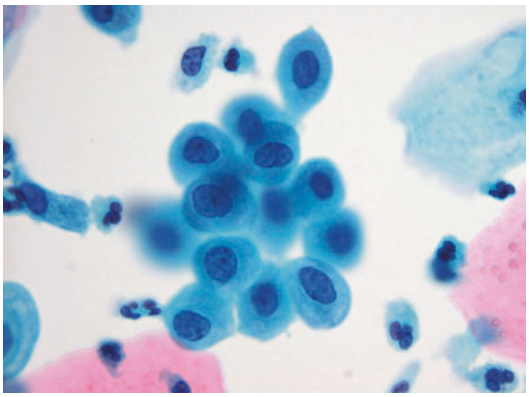
The dynamic process of squamous metaplasia is difficult to capture in a single cervical biopsy. Depending on the stage of the metaplastic process at which a biopsy is taken, the morphological appearances will range from the earliest stages of reserve cell hyperplasia to the point at which mature squamous metaplastic epithelium is indistinguishable from native squamous epithelium. Occasionally, mature squamous metaplastic epithelium overlies several underlying endocervical crypts, causing the development of occlusion cysts known as Nabothian follicles.

Cytology

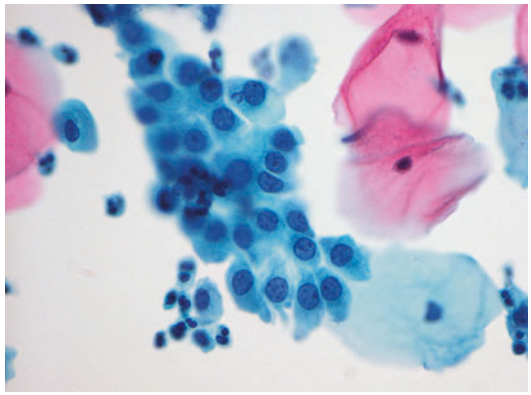
The cytological features of squamous metaplasia recapitulate the spectrum of morphological changes observed histologically. Very early squamous metaplasia closely resembles reserve cell hyperplasia, except perhaps that the cells have a slightly lower nucleocytoplasmic ratio than reserve cells (Fig. 1). Immature squamous metaplastic cells possess densely cyanophilic or polychromatic cytoplasm which becomes more abundant in the mature stages of the metaplastic process. Nuclei are usually round to oval with finely granular vesicular chromatin and smooth nuclear membrane (Fig. 2). One or two small nucleoli may be visible, reflecting the high metabolic activity of such epithelium. Although squamous metaplastic cells can often be seen as in isolation, more often they form cohesive epithelial sheets without significant overlap of cells or nuclei (Fig. 3). The sheets of cells tend to form a mosaic pattern and on high magnification desmosomal intercellular connections may be visible. Perhaps the most characteristic and best known feature of squamous metaplastic cells is the "spider" morphology, in which the cells appear to possess long, slender appendages (Fig. 4). No doubt this appearance is a result of the gradual specialization of the cells into mature squamous-type epithelium.



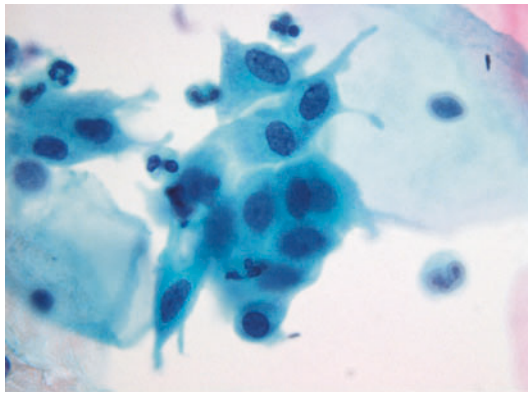
Squamous Metaplasia, Cytological Findings, Fig. 1 Immature squamous metaplastic cells (Pap stain)



Squamous Metaplasia, Cytological Findings, Fig. 2 Maturing squamous metaplastic cells (Pap stain)



Squamous Metaplasia, Cytological Findings, Fig. 3 Cohesive sheet of maturing squamous metaplastic cells (Pap stain)

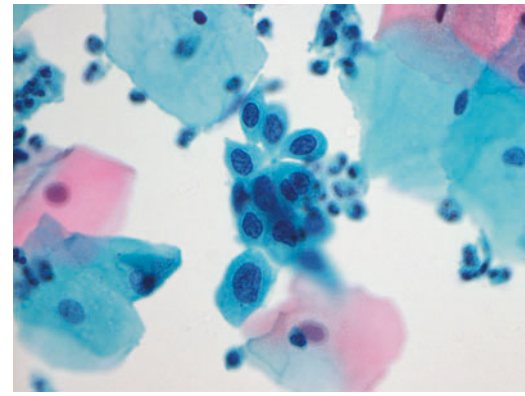


Squamous Metaplasia, Cytological Findings, Fig. 4 "Spider" morphology of squamous metaplastic cells (Pap stain)

The "spider" appearance of squamous metaplastic cells is less prominent in liquid-based cytology preparations compared with conventional slides.

Differential Diagnosis

Squamous metaplasia is a notorious mimic of neoplastic change, particularly when there are superimposed reactive cellular changes. In these circumstances, squamous metaplastic cells may have a moderately high nucleocytoplasmic ratio and mildly hyperchromatic granular chromatin. Slight irregularities in the nuclear membrane can make the distinction from high-grade squamous intraepithelial lesion quite difficult. Cytologically, the flat and uniform architecture of squamous



Squamous Metaplasia, Cytological Findings, Fig. 5 HSIL resembling immature squamous metaplastic cells (Pap stain)

metaplastic cell groups helps to distinguish them from the disorganized three-dimensional cell groups that are more characteristic of high-grade neoplasia (Fig. 5).

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Squamous Papilloma of the Orbit and Ocular Adnexa, Cytological Findings

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Description

Squamous papilloma is the most frequent benign tumor of the eyelid. It may be sessile or pedunculated and composed of projections of acanthotic and parakeratosic epithelium arising

from a fibrovascular stalk. Cells may show atypical nuclei with koilocytotic modifications, as observed in HPV infections.

Cross-References

- Adnexal Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Basal Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- Benign, Nonneoplastic Lesions and Inflammatory Process of the Orbit, Cytological Findings
- Conjunctiva Cytology, General Aspects
- Conjunctival Inflammatory Lesions, Cytological Findings
- Conjunctival Lymphoma, Cytological Findings
- Conjunctival Melanocytic Tumors, Cytological Findings
- Conjunctival Papilloma, Cytological Findings
- Conjunctival Squamous Cell Carcinoma, Cytological Findings
- Cornea Cytology
- Cytology of the Orbit and Ocular Adnexa
- Eyelids Cytology, General Aspects
- Inflammatory Pseudo Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Lacrimal Glands Malignant Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Malignant Melanoma of the Orbit and Ocular Adnexa, Cytological Findings
- Meningioma, Cytological Findings
- Orbit Cytology, General Aspects
- Orbital Cysts of the Orbit and Ocular Adnexa, Cytological Findings
- Orbital Malignant Lymphoma of the Orbit and Ocular Adnexa, Cytological Findings
- Orbital Metastases of the Orbit and Ocular Adnexa, Cytological Findings
- Orbital Soft Tissue Tumors of the Orbit and Ocular Adnexa, Cytological Findings
- Pleomorphic Adenoma of the Orbit and Ocular Adnexa, Cytological Findings
- Sebaceous Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings
- Squamous Cell Carcinoma of the Orbit and Ocular Adnexa, Cytological Findings

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Subareolar Abscess, Cytological Findings

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Synonyms

Recurrent subareolar abscess; Zuska's disease

Definition

Recurrent subareolar abscess is a chronic inflammatory disease characterized by abscess formation at the base of the nipple. The injury can progress with the formation of fistulous tracts and partial healing. It is believed that squamous metaplasia of columnar epithelial cells of the lactiferous duct may be the cause of subareolar abscess. Keratin plugs could obstruct the duct, leading to its dilatation, rupture, and inflammation.

Clinical Features

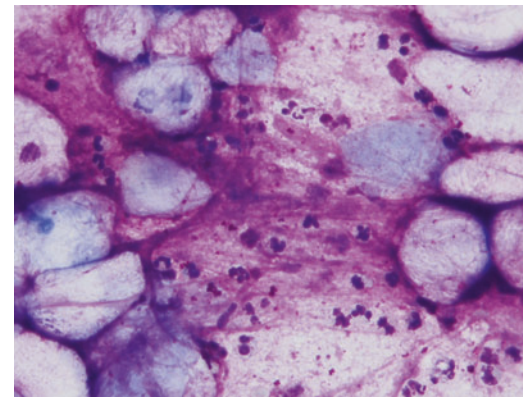
- **Incidence**
  Subareolar breast abscesses usually occur in younger or middle-aged women who are not currently breast-feeding. Because subareolar abscess occurs predominantly in smokers, it is believed that the chronic action of tobacco may alter the epithelium of the lactiferous sinuses.
- **Age**
  Younger or middle-aged women.
- **Sex**
  The higher evidence is in female patients, but it was described in man.
- **Site**
  Base of the nipple.
- **Treatment/Outcome**
  The abscess can require drainage and treatment with antibiotics. If the symptoms come back repeatedly after initially clearing up, surgical treatment is indicated.

Macroscopy

[Grossly signs of inflammatory disease such as dolor, calor, and loss of function. Usually associated with a pseudo-mass formation with pain.]

Microscopy

Aspirates contain a mixture of neutrophils, lymphocytes, histiocytes, anucleate squamous cells, parakeratotic cells, granulation tissue, and multinucleated giant cells of foreign body type with squamous cells and cholesterol crystals (Fig. 1).



Subareolar Abscess, Cytological Findings, Fig. 1 Subareolar abscess. The smears show the presence of inflammatory cells and anucleated squamous cells (Diff-Quik stain)

Immunophenotype

No specific immunophenotype features have been reported.

Molecular Features

No specific molecular features have been reported.

Differential Diagnosis

The main differential diagnosis is with a ruptured epidermoid cyst that is often located peripherally in the breast without relation to the nipple.

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Systemic Disease and Effusions, Cytological Findings

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Synonyms

Lupus effusion; Rheumatoid effusion

Definition

Pleural involvement in systemic diseases is usually a manifestation of disease occurring at other body sites. Approximately 1% of pleural effusions occur in association with various connective tissue diseases and vasculitis. The most common of these systemic diseases that present with effusions and have characteristic cytological features are rheumatoid arthritis and systemic lupus erythematosus (Ferreiro et al. 2011).

Cross-References

- ▶ Rheumatoid Arthritis (RA), Effusions Associated with
- ▶ Systemic Lupus Erythematosus (SLE), Effusions Associated with

References and Further Reading

Ferreiro, L., Alvarez-Dobano, J. M., & Valdes, L. (2011). Systemic diseases and the pleura. *Archivos De Bronconeumologia, 47*(7), 361–370.

Systemic Lupus Erythematosus (SLE), Effusions Associated with

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Synonyms

Lupus-associated effusions

Clinical Features

Pleural, peritoneal, and pericardial effusions can develop in patients with SLE. Pleural effusion is by far the most common of the three sites and is primarily due to serositis (pleuritis) (Ferreiro et al. 2011). Effusion in patients with SLE can also be secondary to renal failure, pulmonary embolism, infection, or congestive heart failure (Kriegel et al. 2009; Naylor 1992). Lupus pleuritis is thought to result from immune-complex deposition, complement activation, and direct binding of anti-dsDNA antibodies to mesothelium (Kriegel et al. 2009).

Macroscopy

The pleural effusion is usually small to moderate (400–1,000 mL) and bilateral (Kriegel et al. 2009; Breuer et al. 2005; Wan 2008).

Microscopy

SLE effusion is characterized by an exudate of 230–15,000 leucocytes/μL which is usually neutrophil predominant but may become lymphocytic with time (Kriegel et al. 2009; Breuer et al. 2005; Wan 2008). The pleural fluid glucose

concentration is usually >60 mg/dL and the PH is >7.35 (Wan 2008). Antinuclear antibodies and immune complexes cause the nuclei of dead and injured cells to become denatured and homogenized. The round, dense, homogenous, cyanophilic body about the size of a neutrophil, consisting of altered nuclear material with a small cytoplasmic component, is referred to as a hematoxylin body (Naylor 1992). Hematoxylin bodies are thus referred to because they stain blue with the usual cytologic stains including the Papanicolaou stain, the Wright Giemsa stain and its modifications (Diff-Quick), and Hematoxylin and Eosin. Hematoxylin bodies may occur both free and ingested, singly and multiply (Fazio et al. 1998). When a hematoxylin body becomes phagocytosed, the phagocytic cell (usually a neutrophil, occasionally an eosinophil or a macrophage) with its engulfed contents is known as lupus erythematosus (LE) cell (Naylor 1992) (Fig. 1). When the ingested hematoxylin bodies flatten out the phagocyte nucleus at the cell periphery and only one hematoxylin body is present per cell, the LE cell may resemble a signet ring cell of adenocarcinoma (Fazio et al. 1998) (Fig. 2). Occasional neutrophils and macrophages phagocytose better preserved, non-homogenous nuclear material of other cells, and are referred to as Tart cells or pseudo-LE cells (Naylor 1992; Fazio et al. 1998) (Fig. 3).

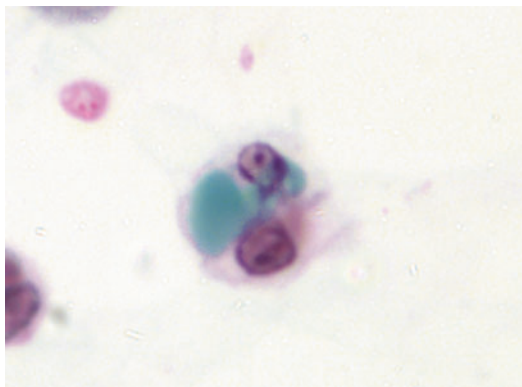
The presence of either anti-DNA antibodies or LE cells in the pleural fluid is considered diagnostic of SLE (Wan 2008). However, the LE cell is not specific for SLE (Fazio et al. 1998; Chao et al. 1997).

Molecular Features

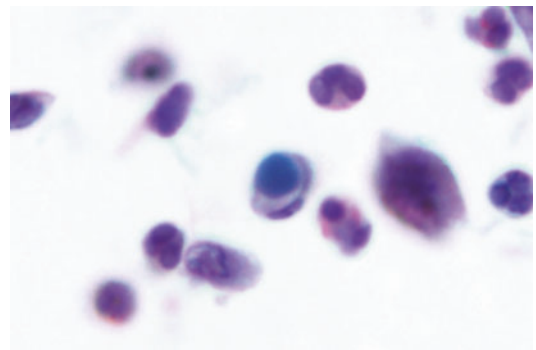
The most helpful features to distinguish lupus pleuritis from other causes of effusions are immunological such as reduced levels of complement and the presence of immune complexes, ANA, and LE cells (Wan 2008).

Differential Diagnosis

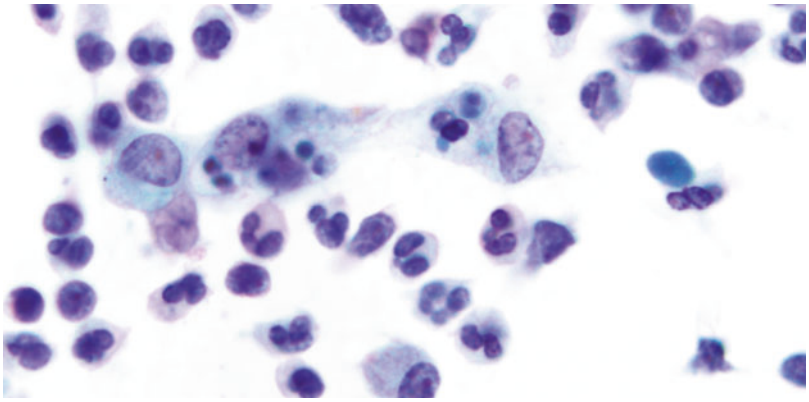
LE cell is not specific for SLE and has been described in patients taking drugs associated with a lupus-like syndrome, such as procainamide, hydralazin, and isoniazid (Fazio et al. 1998), as well as in occasional pleural effusions from patients with cancer but without SLE (Chao et al. 1997).



Systemic Lupus Erythematosus (SLE), Effusions Associated with, Fig. 1 High-power Papanicolaou stain demonstrates an LE cell. A leukocyte contains a homogenized nuclear material also known as Hematoxylin body (Pap stain)



Systemic Lupus Erythematosus (SLE), Effusions Associated with, Fig. 2 High-power Papanicolaou stain demonstrates an LE cell mimicking a signet ring cell as the nucleus of a macrophage becomes flattened by the Hematoxylin body (Pap stain)



Systemic Lupus Erythematosus (SLE), Effusions Associated with, Fig. 3 High-power Papanicolaou stain demonstrates both LE cell (*left*) and Tart cell (*center*). The macrophage contains several phagocytized nuclei that show some degree of preservation compared to the neighboring LE cells (Pap stain)

Cross-References

- ▶ Rheumatoid Arthritis (RA), Effusions Associated with
- ▶ Systemic Disease and Effusions, Cytological Findings

References and Further Reading

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Thymomas, Cytological Findings

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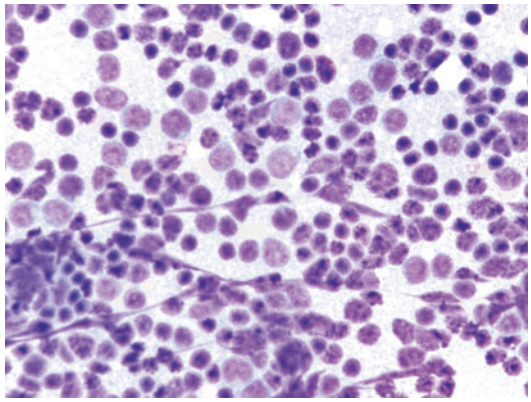
Microscopy

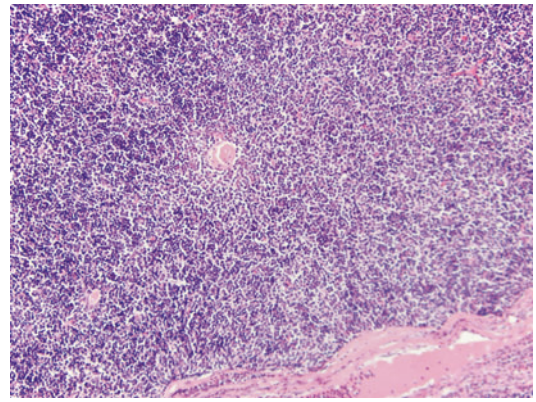
The cytologic features of thymoma FNA specimens are highly variable. However, in general, two cell types must be seen for diagnosis: epithelial cells and lymphocytes. The epithelial cells are usually either spindled with elongated oval nuclei, fine chromatin and inconspicuous nucleoli (WHO Type A), or epithelioid with uniform round-to-oval nuclei, fine chromatin, and variably sized nucleoli (WHO Type B). In cellular aspirates, the epithelial cell component should be readily appreciated, with numerous cohesive clusters of cells, as well as single epithelioid or spindle cells. In many cases, the lymphoid component predominates, consisting of a polymorphous population of lymphocytes and sometimes germinal center elements, such as tingible-body macrophages and follicular center cells (Figs. 1–3), thus mimicking the cytological features of a reactive lymph node (Demay 2012). Hassall's corpuscles are extremely uncommon in thymoma, and one should not expect to encounter them in smears (Wakely 2005). The epithelial cells seen in thymomas generally express EMA and CK 7, and are negative for CK 20. Also, the epithelial cells of WHO Type A thymomas often express CD20. For WHO Type AB and Type B thymomas, the background lymphocytes have been shown to express CD3, CD5, CD20, CD1a, and CD99, with focal expression of CD57 (Alexiev et al. 2007).

Thymic carcinomas (WHO Type C) can be broadly subdivided into non-neuroendocrine and neuroendocrine carcinomas. The most commonly seen non-neuroendocrine thymic carcinomas are squamous cell carcinomas and variants thereof (e.g., basaloid carcinoma, lymphoepithelioma-like carcinoma). The cytological features of squamous cell carcinoma and variants are described elsewhere (Figs. 3–9). The neuroendocrine thymic tumors are classified in the same manner as pulmonary neuroendocrine tumors – ranging from

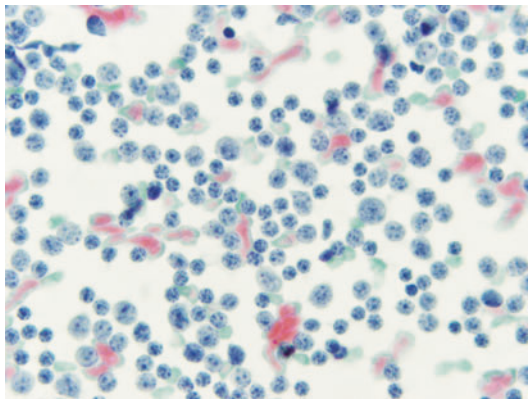
F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4



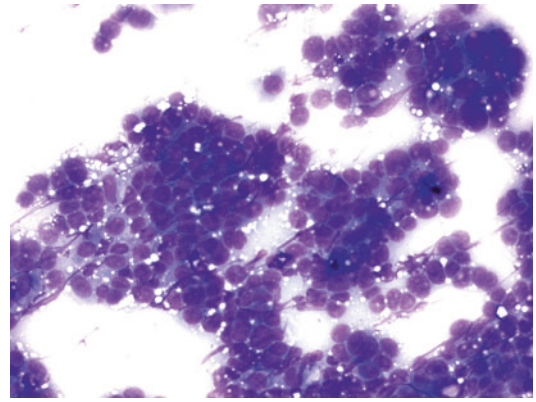
Thymomas, Cytological Findings, Fig. 1 High power Diff-Quik stain demonstrating a lymphocyte predominant thymoma (Type 1B). Notice the polymorphous population of lymphocytes mimicking a reactive lymph node (Diff-Quik stain)



Thymomas, Cytological Findings, Fig. 3 Medium power H&E stain demonstrating the correlating surgical resection of the same case (Type 1B)



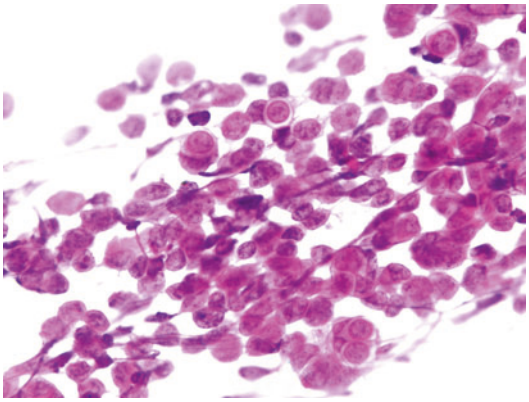
Thymomas, Cytological Findings, Fig. 2 High power Papanicolaou stain demonstrating the lymphocyte predominant thymoma (Type 1B) (Pap stain)



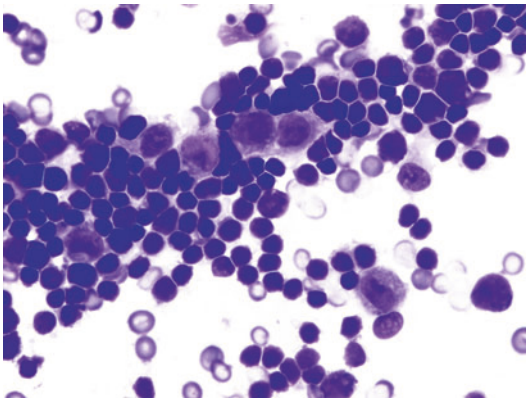
Thymomas, Cytological Findings, Fig. 4 High power Diff-Quik stain demonstrating Type C thymoma (thymic carcinoma). The smear contains sheets of poorly differentiated epithelial cells with rare sprinkled lymphocytes. Background of lymphocytes noted on other areas of the smear (Diff-Quik stain)

typical carcinoid to atypical carcinoid to small cell carcinoma and large cell neuroendocrine carcinoma. Again, those cytological features are described elsewhere.

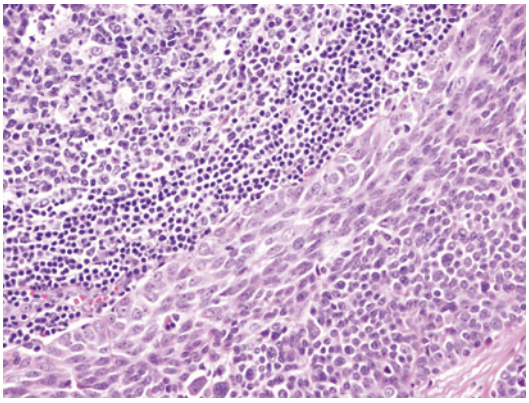
On cytology, the main differential diagnoses for thymoma include seminoma, carcinoma, and lymphoma. In addition, thymic hyperplasia may be a possibility, but it is essentially



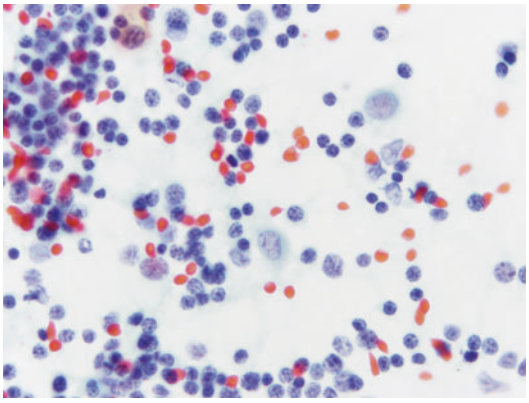
Thymomas, Cytological Findings, Fig. 5 High power Papanicolaou stain demonstrating the same poorly differentiated epithelial cell population with admixed lymphocytes (Pap stain)



Thymomas, Cytological Findings, Fig. 7 High power Diff-Quik stain demonstrating invasive thymic carcinoma with numerous lymphocytes admixed with few atypical epithelial cells (Diff Quik stain)

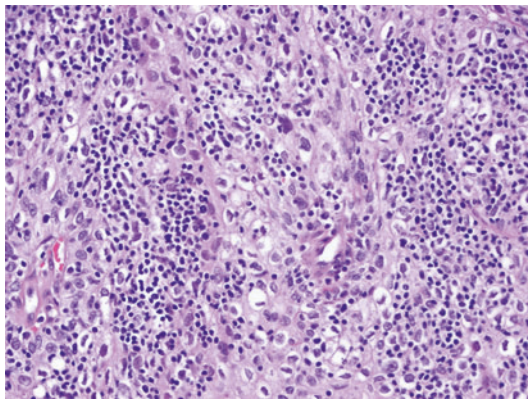


Thymomas, Cytological Findings, Fig. 6 Medium power H&E stain demonstrating the correlating surgical resection of the same case (Type C) (H&E stain)



Thymomas, Cytological Findings, Fig. 8 High power Papanicolaou stain demonstrating the lymphocytes admixed with few highly atypical small epithelial cells with high nuclear to cytoplasmic ratio and irregular nuclear membrane (Pap stain)

indistinguishable from thymoma. Seminoma, with its usual lymphocytic background, may be particularly difficult to differentiate from thymoma (Chhieng et al. 2000). While immunohistochemistry may be helpful to distinguish thymoma from other neoplasms, it should be emphasized that the cytological diagnosis of thymoma is based on the presence of a dual population of epithelial cells and lymphocytes in the correct clinical and radiologic settings (Wakely 2008).



Thymomas, Cytological Findings, Fig. 9 Medium power H&E stain demonstrating the correlating surgical resection of the same case (invasive thymic carcinoma) (H&E stain)

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Toxoplasmosis, Cytological Findings

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Synonyms

Piringer-Kuchynka lymphadenitis

Definition

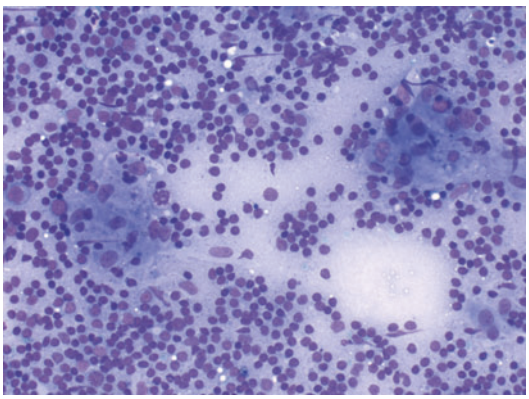
Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii*. The primary host is the felid family (cat), but the parasite infects many animal and humans. The parasite spreads by the ingestion of infected meat or feces of an infected animal or by vertical transmission from mother to fetus.

Primary infection in adolescence and adulthood is usually asymptomatic, but a febrile illness with cervical lymphadenopathy may occur.

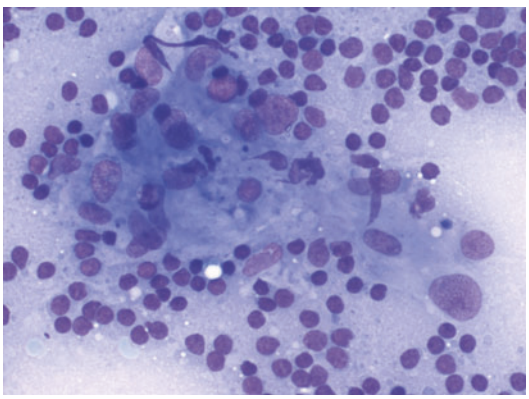
Clinical Features

- **Incidence**
  From one-third to half of the world's human population is estimated to carry a *Toxoplasma* infection. The prevalence of the infection increases with age and the proportion of people infected by *T. gondii* is estimated by serology. The sero-prevalence varies, depending on the intensity of exposure to domestic cats, social habits of eating raw or undercooked meat, and on climate (dry soils favor low prevalence), 90% of people are infected in Paris, about 50% in USA and UK.
- **Age**
  Adults but all ages can be infected.
- **Sex**
  No sex predilection.
- **Site**
  Lymphadenopathy is the most frequent clinical manifestation of acute acquired infection with *Toxoplasma* in the immunocompetent individual. Toxoplasmic lymphadenitis most frequently involved a solitary lymph node in the head and neck regions, without systemic symptoms or extranodal disease and with a benign clinical course. However, serious extranodal disease can occur including myocarditis, pneumonitis, encephalitis, chorioretinitis, and transmission of the infection to the fetus.

- **Treatment**
  Treatment is often recommended only for patients with depleted immunoresponse, such as HIV with CD4 count under 200. A combination of pyrimethamine, sulfadiazine, and leucovorin is often used.
- **Outcome**
  Most patients will respond to combination therapy. However in utero infection will lead to irreversible fetal damage of the fetus.



Toxoplasmosis, Cytological Findings, Fig. 1 Toxoplasmosis: FNA smear from an infected lymph node. A reactive lymphoid cell population with two microgranulomas. MGG



Toxoplasmosis, Cytological Findings, Fig. 2 Toxoplasmosis: Higher magnification of a microgranuloma. MGG

Microscopy: Cytological Findings

Lymph node aspirates consist of mixed lymphoid cells with the presence of follicular center cells and small mature lymphocytes. Scattered epithelioid histiocyte with tendency of microgranuloma formation is the characteristic finding that suggests *Toxoplasma* infection (Figs. 1 and 2). No necrosis, suppurative changes or giant cells are present. Cysts are rarely seen.

Immunophenotype

Commercially available antibodies exist.

Molecular Features

Toxoplasma can be detected using PCR.

Differential Diagnosis

HIV-associated lymphadenopathy, early syphilis lymphadenopathy, and leishmaniasis lymphadenopathy.

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Tuberculous Effusions

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Definition

Exudative effusions occurring in patients suffering from tuberculosis infection and exhibiting the characteristic Lymphocytosis.

Clinical Features (Light 2010)

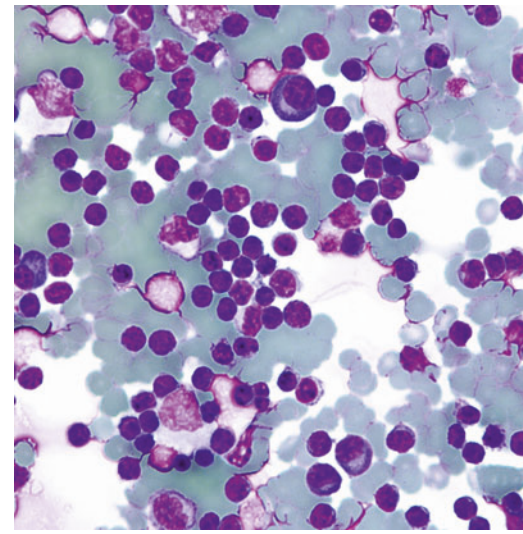
- **Incidence**
  Tuberculosis is a leading cause of pleural effusions in some countries. The percentage of patients with TB who present with effusions is 20–25% in countries where it is prevalent and it is higher in patients who are immunocompromised.
- **Age**
  Patients with TB pleuritis that results in effusion tend to be younger thatn those with parenchymal TB with a mean age of 30 years.
- **Sex**
  TB effusions tend to occur in males who constitute about 68% of cases.
- **Site**
  Usually unilateral and of any size.
- **Treatment**
  The patient should receive the standard treatment for TB. Without treatment the effusion will resolve spontaneously but active tuberculosis frequently recur at a later date.
- **Outcome**
  Effusion should resolve with the treatment of the underlying pleuritis

Macroscopy

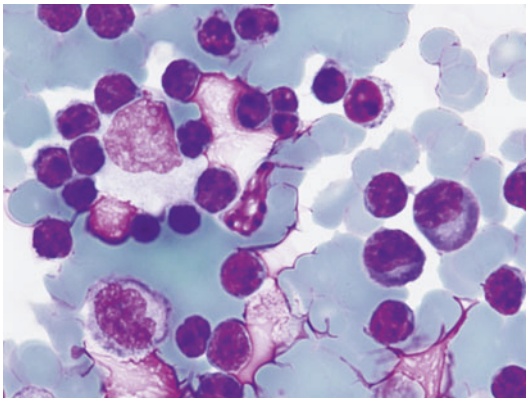
Tuberculous effusions grossly appear turbid, yellow or green, and often with a metallic sheen (DeMay 2012).

Microscopy

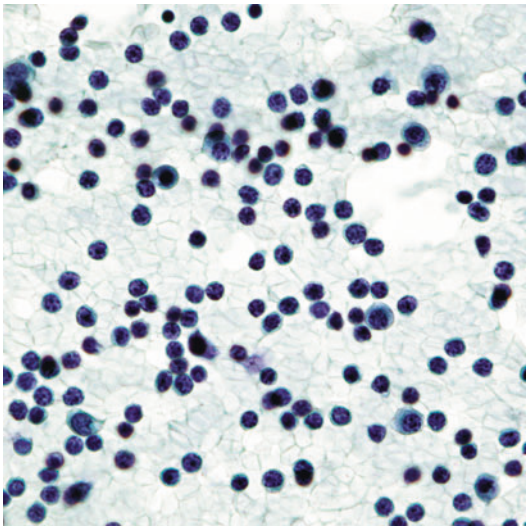
Microscopically, tuberculous pleural effusions are characterized by lymphocytosis instead of caseating granulomatous reaction as is seen in tissue biopsies (Ellison et al. 1998). In addition, the inflammation causes fibrinous destruction of the mesothelial lining, resulting in a marked reduction of exfoliated mesothelial cells (Ellison et al. 1998). In fact, this reduction is so dramatic that a finding of mesothelial cells encompassing



Tuberculous Effusions, Fig. 1 Medium-power Diff-Quik stain demonstrating a predominantly lymphoid population of lymphocytes and scattered plasma cells. Notice the absence of mesothelial cells (Curtsey of Vicki J. Schnadig, M.D., University of Texas medical Center, Galviston, TX) (Diff-Quik stain)

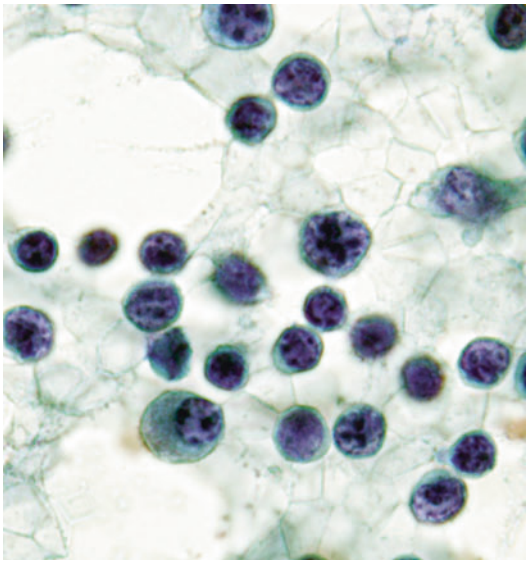


Tuberculous Effusions, Fig. 2 High-power Diff-Quik stain demonstrating the lymphocytes in a tuberculous effusion (Curtsey of Vicki J. Schnadig, M.D., University of Texas medical Center, Galviston, TX) (Diff-Quik stain)



Tuberculous Effusions, Fig. 3 Medium-power Papanicolaou stain demonstrating the lymphoplasmacytic infiltrate in a tuberculous effusion (Curtsey of Vicki J. Schnadig, M.D., University of Texas medical Center, Galviston, TX) (Pap stain)

greater than 10% of the total cell population of a fluid, virtually, excludes the diagnosis of tuberculosis (Ellison et al. 1998). The lymphocytosis may appear monotonous with enlarged, hyperchromatic, and irregular nuclei raising concerns for lymphoma (Figs. 1–4). In these cases, flow cytometry or immunohistochemistry can be used



Tuberculous Effusions, Fig. 4 High-power Papanicolaou stain demonstrating the lymphocytes in a tuberculous effusion (Curtsey of Pradoh k.Gupta, M.D. University of Pennsylvania, Philadelphia PA) (Pap stain)

to exclude lymphoma as the majority of lymphocytes in tuberculosis will be T cells and most lymphomas will be of B cell lineage (Ellison et al. 1998; Okamoto et al. 2005). Macrophages may still be seen in tuberculous effusions; however, neutrophils and eosinophils are not usually present (DeMay 2012).

HIV + patients often have an atypical presentation. Mycobacterial pleuritis may actually result in a proliferation of mesothelial cells in addition to the lymphocytosis causing greater than 10% of the population of cells to be mesothelial cell in origin (Ellison et al. 1998; Okamoto et al. 2005). A culture will be necessary in these cases to exclude tuberculosis.

Special Studies

Culture remains the gold standard to confirm the diagnosis of tuberculous effusions. Special stains, such as an acid fast stain or FITE stain, remain poor at identifying mycobacterial organisms in fluids even in HIV + patients or those with positive cultures.

Immunophenotype

Immunohistochemical stains can show CD3-positive T cells comprising 80% or more of the lymphocyte population (DeMay 2012; Ellison et al. 1998).

Molecular Features

The detection of mycobacterial-specific DNA by polymerase chain reaction can be used to document the diagnosis of tuberculosis (Okamoto et al. 2005).

Differential Diagnosis

The lymphocytosis in tuberculous effusions often shows lymphocyte clumping due to the fibrinous debris. This will not be apparent in effusions due to lymphoma. Although the lymphocytosis often is monomorphic and exhibits reactive atypia often resembling lymphoma, flow cytometry will aid significantly in this diagnosis.

Long-standing inflammatory conditions, chylous effusions from lymphatic obstruction, sarcoidosis, and effusions following cardiac surgery, all should contain greater than 10% mesothelial cells and even reactive mesothelial cell hypertrophy.

Malignant pleural effusions will contain a two-cell population including mesothelial cells and malignant cells in addition to lymphocytes.

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Undifferentiated Carcinoma of the Pancreas, Cytological Aspects

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Synonyms

Anaplastic carcinoma; Giant cell carcinoma; Pleomorphic carcinoma; Pleomorphic large/giant cell carcinoma; Sarcomatoid carcinoma

Definition

Undifferentiated (anaplastic) carcinoma of the pancreas is an aggressive variant of ductal adenocarcinoma characterized by large pleomorphic tumor cells with a variable spindle-cell component (Kloppel et al. 2000).

Clinical Features

- **Incidence**
  Undifferentiated carcinoma of the pancreas represents 2–7% of pancreatic tumors (Paal et al. 2001; Tschang et al. 1977).
- **Age**
  Undifferentiated carcinoma of the pancreas tends to occur in older patients; in two case series, the mean and median ages of patients are both in the seventh decade (Paal et al. 2001; Tschang et al. 1977).
- **Sex**
  Undifferentiated carcinoma of the pancreas is more common in men, with a male:female ratio of 2.5:1 to 4:1 (Paal et al. 2001; Tschang et al. 1977).
- **Site**
  It is unclear whether undifferentiated carcinoma shows a significant predilection for a particular part of the pancreas; cases involving the head, body, tail, and the entire pancreas have been reported (Paal et al. 2001; Tschang et al. 1977).
- **Treatment**
  Due to the aggressiveness of the tumor and advanced stage at which most patients present, surgical resection and radiation therapy for undifferentiated carcinoma of the pancreas are often unsuccessful (Chadha et al. 2004; Kubo et al. 2000).
- **Outcome**
  Undifferentiated carcinoma of the pancreas is one of the most aggressive primary pancreatic cancers, with a 3-year survival rate of less than 3% (Paal et al. 2001).

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
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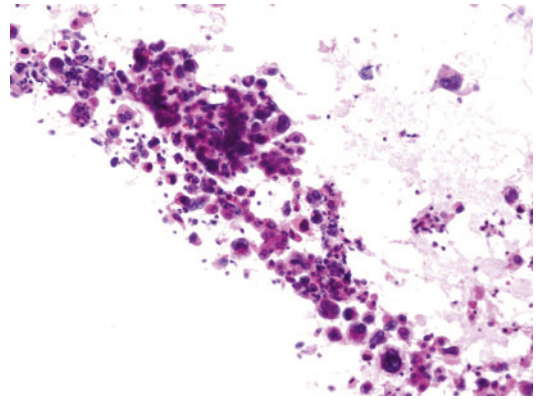
Macroscopy

In the series by Paal et al., tumors ranged from 2.5 to 20 cm (mean, 9.2 cm) in greatest dimension, had a cut surface that ranged from soft/fleshy to firm/rubbery, and showed variable amounts of cystic degeneration and necrotic debris (Paal et al. 2001).

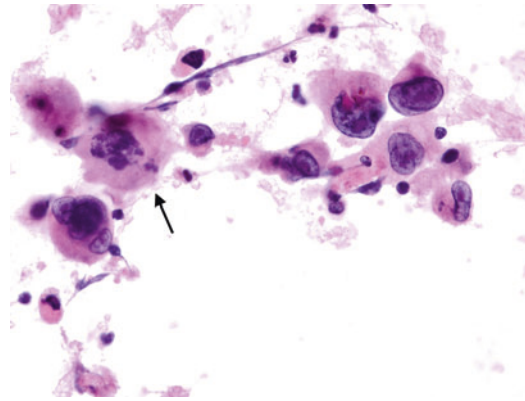
Microscopy

Undifferentiated carcinoma of the pancreas is composed of pleomorphic mononuclear and multinucleated tumor giant cells that demonstrate a poorly cohesive, infiltrative growth pattern. Tumor cells are large, round to polygonal, and have abundant eosinophilic cytoplasm. Nuclei range from round to irregular and angulated, are often eccentrically positioned, and show coarse chromatin. Nucleoli are variably prominent. Tumors are mitotically active, and perineural, lymphatic, and blood vessel invasion are seen in nearly all cases. Foci of more conventional ductal adenocarcinoma may also be present, supporting the epithelial derivation of the pleomorphic tumor cells. Squamoid differentiation has been reported in a few cases (Kloppel et al. 2000; Paal et al. 2001). A subset of undifferentiated carcinomas of the pancreas is predominantly composed of spindle cells in a loose storiform pattern (Paal et al. 2001).

On cytologic specimens, these tumors are poorly cohesive and frequently dispersed as single cells (Fig. 1). Tumor cells are markedly pleomorphic with one or more large, hyperchromatic, eccentrically positioned, often bizarre-appearing nuclei (Figs. 2–5). Mitotic figures are present (Figs. 2–4), including atypical forms. The anaplastic nuclei of multinucleated tumor cells should be easily distinguished from the bland nuclei of non-neoplastic osteoclast-like giant cells, such as those seen in osteoclast-type giant cell tumor of pancreas. Inflammatory cells and necrotic debris may be present in the background.



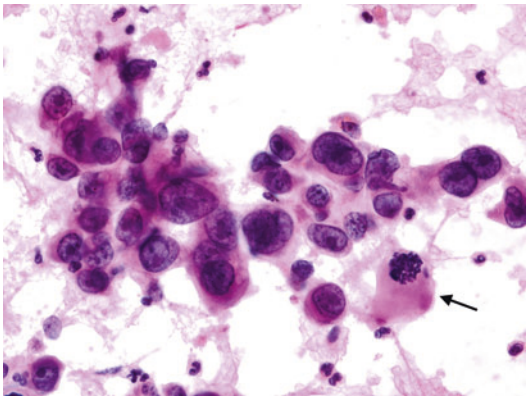
Undifferentiated Carcinoma of the Pancreas, Cytological Aspects, Fig. 1 Fine-needle aspiration of undifferentiated carcinoma of the pancreas that had metastasized to the liver (H&E). Tumor cells are arranged in loosely cohesive clusters and dispersed as single cells. The marked nuclear pleomorphism is evident at low power (Pap stain)



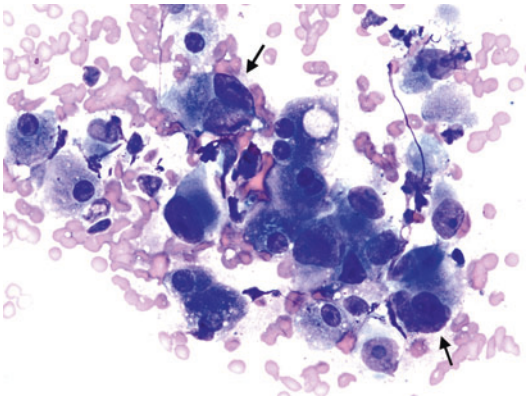
Undifferentiated Carcinoma of the Pancreas, Cytological Aspects, Fig. 2 Undifferentiated carcinoma of the pancreas demonstrating pleomorphic mono- and multinucleated tumor cells with irregular nuclear contours, eccentrically located nuclei, coarse chromatin, and macronucleoli (H&E). Tumor cells have abundant eosinophilic cytoplasm. An atypical mitotic figure is present (*arrow*) (Pap stain)

Immunophenotype

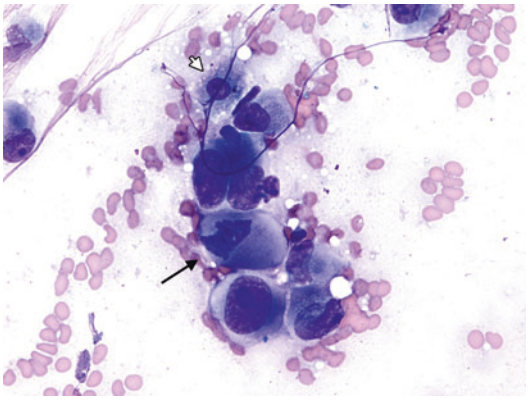
In the series reported by Paal et al., 78% of undifferentiated carcinomas of the pancreas stained for at least one epithelial marker, including cytokeratin AE1/AE3 (14/22 cases), cytokeratin 7 (9/16 cases), and Cam5.2 (8/16 cases) (Paal et al.



Undifferentiated Carcinoma of the Pancreas, Cytological Aspects, Fig. 3 The tumor cells in this field are loosely cohesive and show marked nuclear pleomorphism, coarse chromatin, macronucleoli, and variable amounts of eosinophilic cytoplasm (H&E). A mitotic figure is present (*arrow*)



Undifferentiated Carcinoma of the Pancreas, Cytological Aspects, Fig. 5 Singly dispersed tumor cells with multiple pleomorphic nuclei are seen (*arrows*), admixed with mononucleated tumor cells. Benign hepatocytes are present in the background (Pap stain)



Undifferentiated Carcinoma of the Pancreas, Cytological Aspects, Fig. 4 Fine-needle aspiration of undifferentiated pancreatic carcinoma that had metastasized to the liver (Diff-Quik). A benign hepatocyte (*white arrow*) is admixed with large, singly dispersed tumor cells with pleomorphic nuclei and macronucleoli. A mitotic figure (*black arrow*) is present

2001). Vimentin staining was seen in 18/21 cases, with 15 cases co-expressing vimentin and cytokeratin. Variable staining for CA19-9 (5/18 cases), Dupan2 (7/18 cases), and CEA (8/21 cases) was seen. None of the tumors in their series were immunoreactive for cytokeratin 20, B72.3, chromogranin, or synaptophysin.

Molecular Features

KRAS mutations have been identified in the majority of undifferentiated carcinomas of the pancreas, supporting their derivation from ductal epithelial cells (Hoorens et al. 1998; Paal et al. 2001).

Differential Diagnosis

Undifferentiated carcinoma of the pancreas may exist in a morphologic continuum with osteoclast-type giant cell tumor of the pancreas, with the latter showing less nuclear atypia in the mononuclear and multinuclear cells and better prognosis. The differential diagnosis also includes melanoma, pleomorphic undifferentiated sarcoma, rhabdomyosarcoma, choriocarcinoma, hepatocellular carcinoma, Hodgkin lymphoma, anaplastic large-cell lymphoma, and anaplastic carcinomas of other sites such as lung and thyroid.

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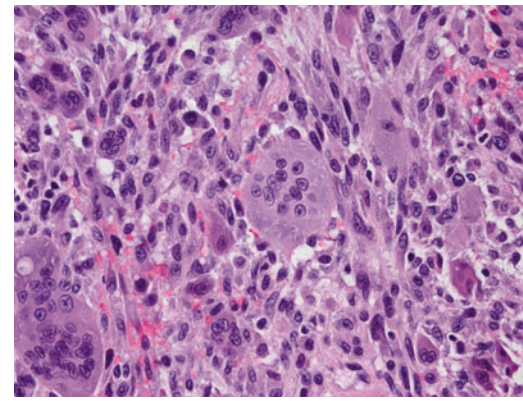

Undifferentiated Carcinoma with Osteoclast-Type Giant Cells of the Pancreas, Cytological Aspects, Fig. 1 H&E-stained section from a total pancreatectomy specimen shows malignant spindled and epithelioid mononuclear cells admixed with osteoclast-like giant cells with centrally clustered, bland, slightly overlapping nuclei (H&E stain)

Undifferentiated Carcinoma with Osteoclast-Type Giant Cells of the Pancreas, Cytological Aspects

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Synonyms

Giant cell carcinoma; Multinuclear giant cell neoplasm; Osteoclastic giant cell tumor of pancreas; Osteoclastoma; Osteoclast-type giant cell tumor of pancreas; Sarcomatoid carcinoma; Variant of pleomorphic carcinoma

Definition

Undifferentiated carcinoma with osteoclast-type giant cells is a variant of pancreatic ductal adenocarcinoma comprised of non-neoplastic osteoclast-type giant cells admixed with a population of variably pleomorphic neoplastic cells (Fig. 1).

Clinical Features

- **Incidence**
  Undifferentiated carcinoma with osteoclast-type giant cells is rare and reported to comprise less than 1% of exocrine pancreatic tumors (Kloppel et al. 1998).
- **Age**
  Among the reported cases, the tumor most often presents in the sixth to seventh decade of life (Molberg et al. 1998).
- **Sex**
  There appears to be no sex predilection (Molberg et al. 1998).
- **Site**
  Cases of undifferentiated carcinoma with osteoclast-type giant cells have been reported in the pancreas as well as liver, extrahepatic bile ducts, ampulla/periampullary region, and gallbladder (Molberg et al. 1998).

- **Treatment**
  Treatment modalities include surgical resection, chemotherapy, and radiation therapy (Moore et al. 2010).
- **Outcome**
  Although the published survival data varies greatly, patients with undifferentiated carcinoma with osteoclast-type giant cells have been reported to have similar to slightly better prognoses as those with conventional pancreatic adenocarcinoma (Molberg et al. 1998; Moore et al. 2010).

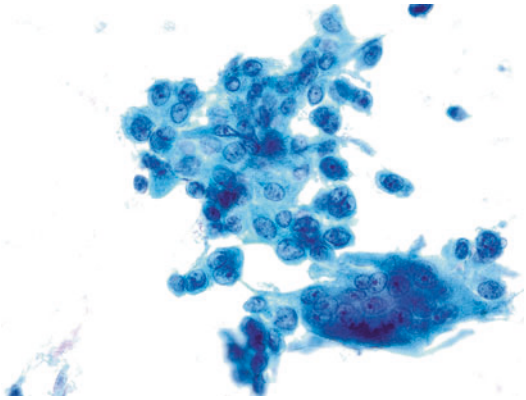
Macroscopy

According to a case series by Molberg et al. of nine patients with undifferentiated carcinoma with osteoclast-type giant cells, resected tumors ranged from 3.5 to 14 cm (average 9 cm) in greatest dimension (Molberg et al. 1998). Tumors were variably cystic, hemorrhagic, and necrotic.

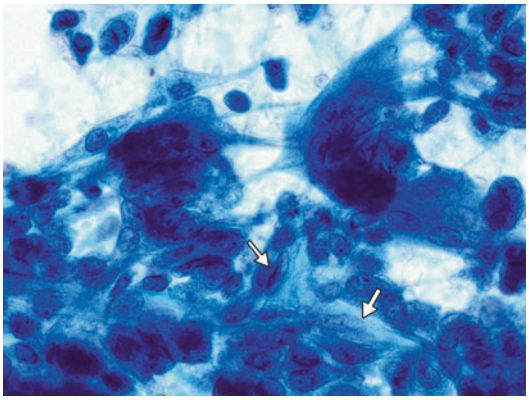
Microscopy

Diagnosis can be made on surgical resection specimens as well as on cytology specimens obtained by endoscopic ultrasound-guided fine needle aspiration (Moore et al. 2010; Layfield and Bentz 2008). Cytologic preparations generally consist of (1) multinucleated osteoclast-type giant cells and (2) mononuclear cells with epithelioid to spindled morphology (Ali et al. 2009; Centeno and Pitman 1999). The osteoclast-type giant cells (Figs. 1, 2, and 3) are non-neoplastic and of histiocytic origin. Cytomorphologically, the giant cells show dense to granular cytoplasm which stain eosinophilic with the hematoxylin/eosin stain, cyanophilic with the Papanicolaou stain, and deep purple with Romanowsky stains. The giant cells have multiple uniform, small, often overlapping, round to oval nuclei with slightly irregular contours. Nucleoli are frequently prominent, and chromatin ranges from vesicular to coarsely granular.

The undifferentiated mononuclear cells are neoplastic, favored to be of ductal origin, and range from epithelioid to spindled morphology. Cells with epithelioid appearance are dyscohesive and polygonal with eccentrically positioned, ovoid nuclei (Fig. 2). Spindled cells form loose fascicular to herringbone architecture and have elongated, blunt-ended nuclei (Fig. 3). The mononuclear cells have foamy to granular cytoplasm and moderate to severe nuclear atypia, with nuclear features ranging from those similar to the



Undifferentiated Carcinoma with Osteoclast-Type Giant Cells of the Pancreas, Cytological Aspects, Fig. 2 Papanicolaou-stained aspirate smear showing malignant epithelioid cells with mildly atypical nuclei, somewhat resembling the bland nuclei of the osteoclast-type giant cell in the lower right corner of the image (Pap stain)



Undifferentiated Carcinoma with Osteoclast-Type Giant Cells of the Pancreas, Cytological Aspects, Fig. 3 Papanicolaou-stained aspirate smear showing mild to moderately atypical spindled cells (*arrows*) with elongated, blunt-ended nuclei (Pap stain)

osteoclast-type giant cells (Fig. 2) to those that are frankly malignant (large nuclei with irregular contours, and coarse chromatin).

Immunophenotype

By immunohistochemical stains, the non-neoplastic osteoclast-type giant cells are positive for KP1 (CD68) and negative for cytokeratins and p53 (Molberg et al. 1998; Westra et al. 1998). The neoplastic epithelioid and spindled cells are variably positive for cytokeratin (AE1/AE3), cytokeratin 20, and p53. The neoplastic cells are negative for KP1 (CD68) (Molberg et al. 1998; Westra et al. 1998).

Molecular Features

Mutations in codon 12 of *KRAS* have been reported in both the neoplastic mononuclear cells as well as the osteoclast-type giant cells. The detection of oncogenic *KRAS* sequences in the non-neoplastic giant cells was hypothesized to be due to their phagocytosis of *KRAS*-mutant tumor cells (Westra et al. 1998).

Differential Diagnosis

Pleomorphic giant cell carcinoma may exist in a morphologic continuum with undifferentiated carcinoma with osteoclast-type giant cells, with the former showing a higher degree of nuclear atypia in both the multinuclear and mononuclear cells as well as poorer prognosis. The differential diagnosis also includes other pleomorphic malignancies with anaplastic multinucleated cells (including carcinomas, sarcomas, and melanomas) and non-neoplastic conditions with multinucleated giant cells (e.g., tuberculosis, foreign body-type giant cell reaction in the setting of fat necrosis, pancreatic pseudocyst, or pancreatic duct obstruction).

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Urine Specimen Cytology

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Synonyms

Voided urine

Definition

Voided urine is the easiest and the least-expensive method of cytologic analysis of the urinary tract. It provides reliable information on the status of the epithelium of the whole urinary tract encompassing the renal tubules, renal pelves, ureters, urinary bladder, and the urethra and is

particularly useful for detecting high-grade urothelial carcinoma (HGUC) and ▶ urothelial carcinoma in situ (CIS). Urine cytology and cystoscopy are superior to cystoscopy alone in detecting high-grade urothelial bladder cancers as well as upper urinary tract tumors. Voided urine cytology is recommended for all patients at risk for urothelial carcinoma. Voided urines also serve for a first, noninvasive, and inexpensive screening in patients with urinary symptoms including hematuria or for screening of persons who have a history of occupational exposure to aromatic amines or other carcinogens. According to a recent meta-analysis, the sensitivity and specificity of urine cytology is 44% and 96%, respectively. However, it has little utility in the diagnosis or follow-up of low-grade urothelial carcinomas (LGUC) due to the low sensitivity for these tumors. Cells from other carcinomas can also occasionally be found including ▶ renal cell carcinoma and ▶ prostatic adenocarcinoma. Search for ▶ polyomavirus-infected decoy cells or signs of allograft rejections in patients after renal transplantation is another important application of voided urine analysis. In addition, testing for acute inflammation in voided urines after prostate massage is used for the diagnosis of chronic prostatitis. In patients with increased serum PSA due to asymptomatic inflammation, this test may avoid unnecessary prostate biopsies.

Principle

The second morning urine is collected and analyzed for cellular abnormalities.

Methodology

The first morning urine contains the richest population of cells, but they are often poorly preserved. Therefore, it is recommended to use the second morning midstream voided urine and a suitable plastic container that holds about 250 or 300 ml. Fresh urine specimens should be promptly delivered to the cytology laboratory for processing within a few hours. If this is not possible, the voided urine is diluted with an equal volume of 50% ethyl alcohol, optionally with added 2% Carbowax (polyethylene glycol), to preserve the cells for up to 3 days and prevent bacterial overgrowth during transport. Conventional cytospins on coated glass slides are the preferred method of preparation. The slides should be immediately fixed in alcohol and stained according to Papanicolaou. Fluid-based cytology preparations (e.g., ThinPrep®, UroCyte®, or SurePath®) are a valid alternative, although they lead to increased costs without clearly improving the quality as compared to cytospins. Because the cells exfoliate from the urothelium intermittently, it has been proposed that three urine samples should ideally be examined from three consecutive days to ensure that the diagnostic cells are sampled. However, this is not always feasible due to practical reasons.

Quality Aspects

All available fluid should be sent to the laboratory. The details of the nuclear features are critical for an accurate diagnosis. Therefore, immediate fixation of the cytospin specimens is important in order to avoid drying of the cells. Numerous bacteria without acute inflammation indicate secondary bacterial overgrowth due to suboptimal conditions during the transport, such as long delay, high temperature, and no adding of 50% ethanol for preservation. Admixed fresh blood that may obscure the morphology of urothelial cells is not a common problem.

Applications

Noninvasive diagnosis of urothelial carcinoma in patients with hematuria or a history of ▶ urothelial carcinoma

Screening for ▶ polyomavirus-infected cells (decoy cells in patients after renal transplantation)

Diagnosis of prostatitis by voided urine after prostate massage

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Urothelial Carcinoma, Cytological Findings

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Synonyms

Transitional cell carcinoma (term not recommended)

Definition

The bladder is the most common site of urothelial carcinomas, as only 5–10% of all of these tumors occur in the upper urinary tract (ureter and renal pelvis). Urothelial carcinoma is by far the most common histological carcinoma subtype of the urothelial tract (90–95%), whereas squamous cell carcinomas and adenocarcinomas are much less common (about 2%, each). Approximately 70–80% of patients with newly diagnosed bladder cancer present with noninvasive or early invasive disease (i.e., stage pTa, pTis, or pT1). Only 5–10% of these will progress to muscle-invasive disease. Cytology of the urinary tract has successfully been used for initial diagnosis and surveillance of urothelial neoplasias for decades. Nevertheless, the lack of a uniform classification system of urinary cytology has been a main problem, which has led to innumerable local classification systems and major problems in interlaboratory communication. This is further complicated by complex clinical pathological associations due to the fundamentally different clinical significance of the various types of urothelial tumors and the different algorithms for treatment and surveillance. Diagnosis and the interpretation have also constantly been adapted to the changing concept in histopathological classification of urothelial tumors. Methods to obtain cytological materials include voided urines, bladder washings and brushes, or washings of the upper urinary tract consisting of ureter and the renal pelvis. Voided urines are often paucicellular and the cells typically reveal degenerative changes. Instead, washings are much more cellular, and the cells are much better preserved, allowing for more accurate diagnosis. The major strength of urinary cytology is to diagnose high-grade urothelial carcinoma (HGUC) including papillary and solid HGUC and carcinoma in situ (CIS), while detection of the by far less dangerous low-grade urothelial neoplasias is not a priority of cytology.

Clinical Features

- **Incidence**
  Bladder cancer is the most common malignancy in the urinary tract and the 7th most common cancer in men and the 17th in women. Approximately 75% of patients present with non-muscle-invasive bladder cancers (NMIBC) that is either confined to the mucosa (stage pTa, CIS) or the lamina propria (stage pT1). Up to 70% of NMIBC urothelial carcinomas recur, which makes it a chronic disease with long-term follow-up including repeated voided urines, cystoscopies, and bladder washings. The high recurrence rate, the intensive surveillance strategies, and the expensive treatment costs make bladder cancer the cancer with the highest cost per patient.

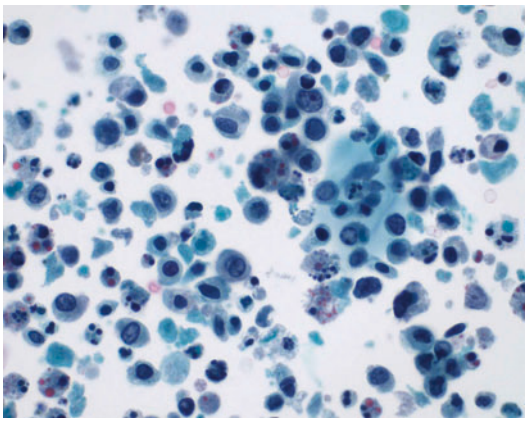
- **Age**
  Urothelial tumors are typically a disease of elderly people. The median ages of patients at the time of the initial diagnosis are 69 years in men and 71 years in women.
- **Sex**
  Bladder cancer is more common in male than in female (the ratio is about 3.5:1). Smoking increases the risk of bladder cancer by a factor of 2–6.
- **Site**
  Urothelial carcinomas are often multifocal due to a cancer field effect of the whole bladder and upper urinary tract or due to intraepithelial spreading of tumor cells. Approximately 5–10% of all recurrences occur in the upper urinary tract.
- **Treatment**
  NMIBC are treated by transurethral resection. In high-risk tumors, this is followed by intravesical treatment with Bacille Calmette–Guérin (BCG). This BCG treatment leads to an immunological reaction against tumor cells and reduces recurrence and progression to muscle-invasive disease. Radical cystoprostatectomy or partical cystectomy is the treatment of choice in patients with muscle-invasive bladder cancer.
- **Outcome**
  The prognosis largely depends on the pathological stage and grade of the disease. Low-grade noninvasive urothelial carcinomas (pTa, low-grade) frequently recur but rarely progress to life-threatening muscle-invasive bladder cancer. The risk of progression increases to up to 30% in carcinomas with infiltration of the lamina propria (pT1). Depending on the stage (pT2 vs. pT3) or nodal status (pN0 vs. pN1), 40–70% of patients with muscle-invasive urothelial carcinoma succumb to the disease despite radical surgery.

Macroscopy

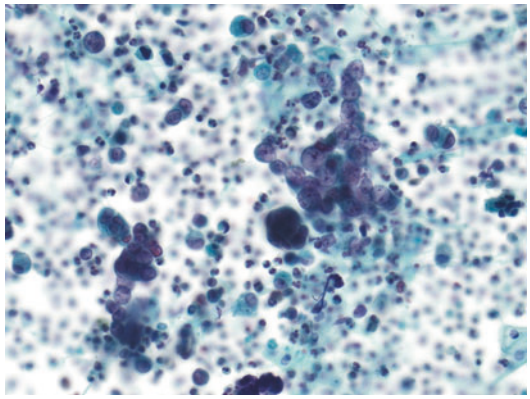
Details on how to process voided urines and bladder washes are described in the entries on bladder washes and ▶ urine specimens. Two cytospin specimens are considered as representative. Fluid-based cytology preparations (e.g., ThinPrep®, UroCyte®, or SurePath®) are a valid alternative, although they lead to increased costs without clearly improving the quality as compared to cytospins. Cytological specimens of the urinary tract should be stained according to Papanicolaou or by hematoxylin–eosin in order to highlight the nuclear details that are critical for cytological grading.

Microscopy

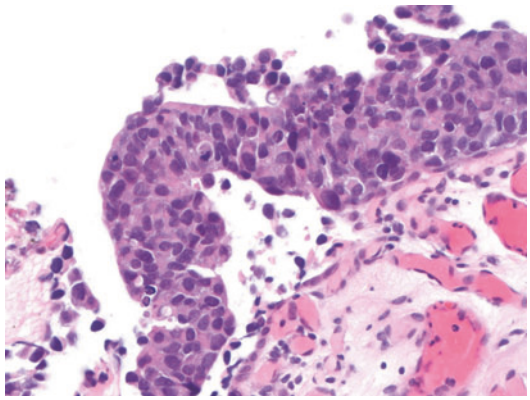
Detection of HGUC is the major strength of cytology, as emphasized in the forthcoming new "Paris system of reporting urinary cytology." This new consensus system aims at reliably diagnosing high-grade UC by the cytological categories of "HGUC" and "atypical urothelial cells, suggestive of HGUC" (AUC-H) from other findings and at identifying rewarding categories for ancillary testing. The cytological diagnosis of high-grade UC and carcinoma in situ (CIS) is straightforward. The mostly discohesive tumor cells catch the eye by their dark, hyperchromatic nuclei, which show high variability in size and shape and an irregular nuclear membrane (Figs. 1, 2, and 3). The chromatin is irregularly structured and usually coarsely granular with or without prominent nucleoli. The nuclei are excentric and the nuclear-cytoplasmic ratio is high. A clear cytological



Urothelial Carcinoma, Cytological Findings, Fig. 1 High-grade urothelial carcinoma. Discohesive atypical cells with hyperchromatic and polymorphic nuclei (Voided urine, Papanicolaou, ×600)



Urothelial Carcinoma, Cytological Findings, Fig. 2 Carcinoma in situ in bladder washing. Tight clusters of highly atypical urothelial cells with polymorphic nuclei, chromatin abnormality, and high N/C ratio. Acute inflammatory background (bladder washing, Papanicolaou, ×400)



Urothelial Carcinoma, Cytological Findings, Fig. 3 Carcinoma in situ, histology. Histology after bladder washing shown in Fig. 2 (biopsy, hematoxylin–eosin, ×400)

diagnosis of flat or papillary low-grade urothelial neoplasia is difficult and often impossible, especially in case of papilloma, papillary urothelial neoplasia with low malignant potential (PUNLMP), and well-differentiated noninvasive urothelial carcinoma (G1 according to WHO 73). The cytological features of low-grade urothelial neoplasia are described in detail in the chapter "► Low-Grade Papillary Urothelial Carcinoma, Cytological Findings" of this Encyclopedia. The diagnosis has low clinical importance, since they are quite harmless and usually visible by cystoscopy. Nevertheless, even in case of low-grade papillary tumors removed by transurethral resection, cytology has a value as it can detect high-grade components or adjacent carcinoma in situ that have been missed by histology. In addition, voided urines allows for detecting UC from upper urinary tract.

Besides classical urothelial carcinoma, there are rare variants defined by special growth patterns or features including the plasmacytoid, micropapillary, and nested variants; urothelial carcinoma with small tubules or microcystic urothelial carcinoma; and others. Across this whole spectrum, the main task of cytology remains the diagnosis of high-grade urothelial carcinoma by general criteria irrespective of variants. Some of the rare variants can be diagnosed or at least be suspected by cytology, though. In case of adenocarcinoma, squamous cell carcinoma, and neuroendocrine carcinomas (including ► small cell carcinoma) of the bladder, the same diagnostic criteria apply as in other organs.

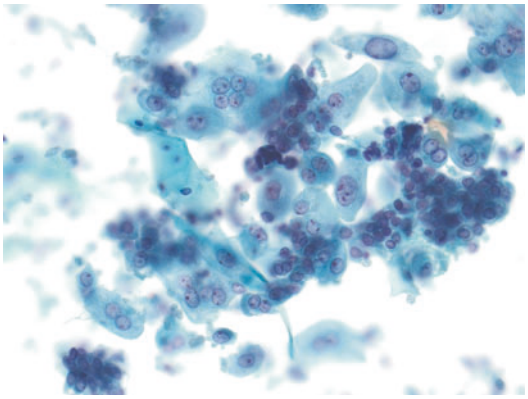
Cytology of the upper urinary tract (UUT, ureter, or renal pelvis) often has a somewhat different appearance than bladder washings. In addition to activated umbrella cells, benign UUT washings contain numerous medium-sized urothelial cells with a high N-C ratio as the urothelial cells in bladder washings. Depending on the physical force of the washing, they may also form balls or papillary clusters. The cytological evaluation of UUT washings is greatly facilitated when bilateral specimens are submitted. Recognizing subtle differences between the two sides can be helpful.

Immunophenotype

Urothelial cells are diffusely positive for cytokeratin 7, p63 and CK5/5, as well as GATA-3. Cytokeratin 20, which mainly stains normal umbrella cells in the benign urothelium, becomes often diffusely positive in urothelial carcinoma. These markers allow for a reliable differentiation between urothelial carcinoma and infiltration or metastasis of an extra-vesical carcinoma in most of the cases in which a classification based on morphology alone is difficult.

Molecular Features

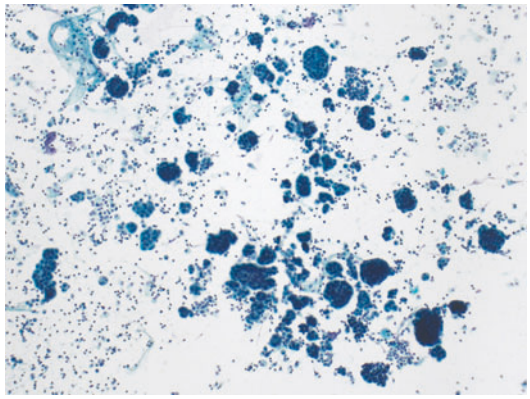
Chromosome 9 alterations are the earliest genetic alterations in urothelial carcinoma with 9p21 deletions (p16 locus) being particularly common. This is followed by chromosomal instability leading to increased chromosome copy numbers of chromosomes, including chromosomes 3, 7, and 17. A multi-target fluorescence in situ hybridization (FISH) assay was developed to detect these alterations (UroVysion, Abbott Molecular Inc., Des Plaines, IL). This FISH assay has a sensitivity of up to 0–100% for the detection of invasive bladder cancer (pT1-4) and a specificity of almost 95%. In the category of low-grade noninvasive bladder cancer (pTa low-grade), FISH increases the sensitivity of cytology from 25% to 60–75%. In the setting of surveillance, FISH can be used to better estimate the risk of recurrence. In daily practice, FISH is most useful for elucidation of equivocal cytology. Equivocal findings are particularly a problem after bacillus Calmette–Guérin treatment of carcinoma in situ, in which even reactive changes can appear most worrisome. ImmunoCyt®/uCyt+ (Scimedx, Denville, NJ, USA), a fluorescent test combining three antibodies, is another encouraging marker to increase the sensitivity of cytology, although the specificity appears to be lower as compared to FISH. *Fibroblast growth factor 3 (FGFR3)* mutations prevail in 70% of non-muscle-invasive bladder cancer and can be used for early detection of recurrences. Promoter hypermethylation of tumor suppressor genes is also common in bladder cancer and DNA methylation of several genes, and gene panels have been reported as diagnostic markers in voided urines and bladder washings. One of the most promising methylation marker panels studied is *TWIST* and *NID2* gene methylation with a sensitivity and specificity of 90% and 93%, respectively. Non-cytology-based markers such as NMP22, BT-STAT, or CYFRA 21-1 may be used as an initial test to better identify patients with an increased risk of urothelial carcinoma, but they suffer from low specificity. According to an ICUD-EAU International Consultation from 2012, none of the many molecular markers has achieved acceptance as a standard diagnostic procedure due to their generally limited specificity. However, there is mounting evidence that FISH and ImmunoCyt® testing might be most rewarding in the category of atypical urine cytology (AUC-US or AUC-H).



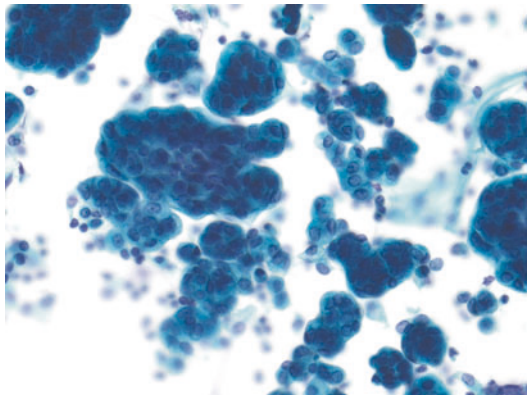
Urothelial Carcinoma, Cytological Findings, Fig. 4 Reactive urothelial cells after BCG treatment. Activated umbrella cells with finely vacuolated cytoplasm and groups of smaller urothelial cells (bladder washing, Papanicolaou, ×400)

Differential Diagnosis

Pronounced reactive changes after intravesical BCG or in patients with urolithiasis are notoriously difficult and can be misinterpreted as HGUC or CIS depending on the experience of the cytologist (Fig. 4). The nuclear details and the nuclear-cytoplasmic ratio are critical for a correct diagnosis. Three-dimensional rounded cell groups or clusters, which are commonly seen in instrumented urinary cytology such as bladder washings and preparations of the upper urinary tract, are often misinterpreted as evidence of low-grade papillary tumors (Figs. 5 and 6). These cell clusters lack relevant atypia and typically contain umbrella cells as a clue to the correct diagnosis. ▶ Decoy cells in patients with rare polyoma virus cystitis can masquerade as carcinoma cells to those with low experience in recognizing such cells. Poorly differentiated urothelial carcinoma might be mimicked by locally advanced, poorly differentiated adenocarcinomas of the prostate with a solid or diffuse growth pattern. Besides clinical history and information on the serum PSA value, prominent nucleoli are



Urothelial Carcinoma, Cytological Findings, Fig. 5 Washing of the renal pelvis with benign cytology. Balls and pseudopapillary clusters of reactive urothelial cells with umbrella cells at the surface (Papanicolaou, ×100)



Urothelial Carcinoma, Cytological Findings, Fig. 6 Washing of the renal pelvis with benign cytology. Higher magnification of Fig. 5 (Papanicolaou, ×400)

a clue towards prostatic adenocarcinoma. A definitive diagnosis can be achieved by a limited immunocytochemical marker panel including cytokeratin 7 and GATA-3 (consistently positive in urothelial carcinoma but negative in prostate cancer) and PSA and ERG (negative in urothelial carcinoma but positive in a large fraction of prostatic adenocarcinomas). Squamous cell carcinomas might originate from advanced cervico-vaginal carcinoma and intestinal-type adenocarcinoma from advanced colorectal or gastric adenocarcinoma.

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V

Viral Infections, Cytological Findings

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Introduction

Definition

Viral infections are among the most common infections in humans. As they are intracellular organisms, it is impossible to identify them visually by light microscopes. Viral infections can be suspected if specific cytopathic effects are noticed on the infected cells. In some viral infections cellular changes may have typical appearance, so cytological diagnosis of the viral infection can be leading one in confirming a final diagnosis (Kocjan et al. 2013; Gray and Kocjan 2010).

Epidemiology

The epidemiology of infectious disease is concerned with the circumstances under which both infections and disease occur in a population and the factors that influence their frequency, spread, and distribution. It is critical to distinguish between infection and disease because the factors that govern their occurrence may be diferent and because infection without disease is common with many viruses (Kaslow et al. 2014).

Diagnosis

Methods used in diagnostic virology are viral culture, electron microscopy, light microscopy (cytology and histology), antigen detection by immunocytochemistry or immunohistochemistry, serology, and molecular techniques.

Cytologic Findings

Cytologic examination for evidence of viral infection can be performed on smears prepared by applying a specimen directly to a microscope slide by scraping the base of skin or mucocutaneous lesions with a scalpel blade; on slides prepared by cytocentrifugation of fluids such as bronchoalveolar lavage fluid, cerebrospinal fluid, or urine; and on touch preps prepared from pieces of unfixed tissue (Knipe and Howley 2013).

Infected cells by viruses often show:

- Inclusion formations. Inclusions are dense, homogenous, often eosinophilic intracellular structures usually consisting of viral particles. They can be found in cytoplasm and/or within nuclei of infected cells. Usually are typical and diagnostic for some viral infection, for

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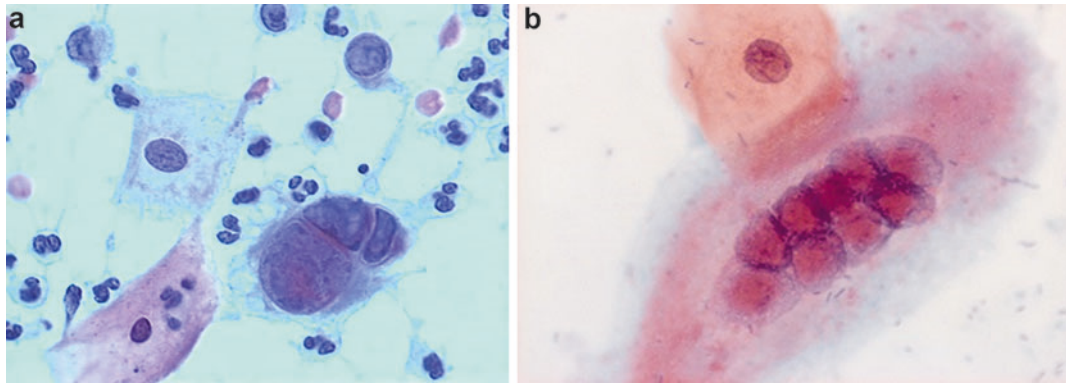


Viral Infections, Cytological Findings, Fig. 1 Herpes simplex virus. (**a**) Giant, multinucleated cell with folding nuclei; (**b**) Giant multinucleated cell resembling foreign-body giant hystiocite (Genital infection, Conventional Pap smear, Papanicolaou ×400)

example, cytomegalovirus (CMV) or herpes simplex virus (HSV) infections.

- Hydropic degeneration, which is a result of the organelle membrane injuries. Sometimes it can be accompanied with the development of the inclusion bodies.
- Cell lysis and necrosis. Cytoplasms of the infected cells become thickened and without usual transparency because of coagulative necrosis induced by some viruses. Sometimes degenerative nuclear changes like fragmentation and lysis of chromatin are visible as is in adenovirus infections.
- Formation of giant cells, which happens because of the alteration in the cell membrane of the infected cells. Result is syntitial and giant forms of infected cells with or without inclusion bodies – like in HSV infection (Fig. 1).
- Sometimes interaction between virus and host cells leads to changes on the surface of the infected cells so they form clumps of cells in different sizes (like in human papilloma virus (HPV) infections), as well as intense reactive changes at the cytoplasm of the cells often shown as parakeratosis or dyskeratosis due to alternated keratin metabolism.

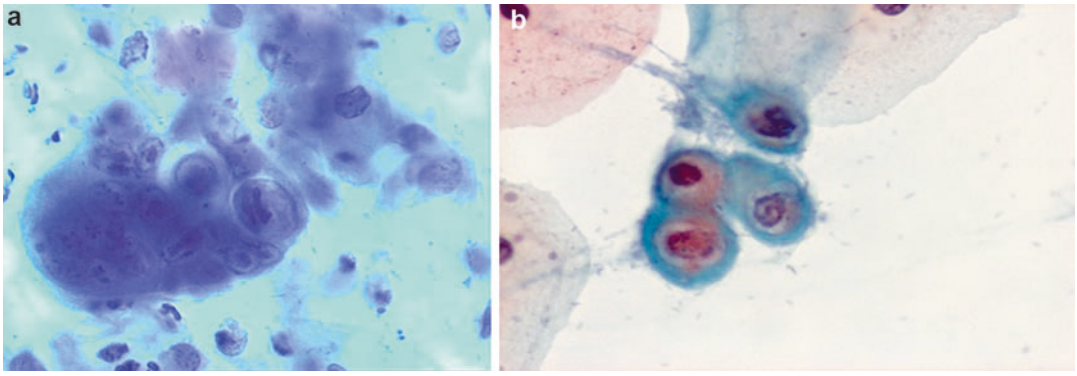
Types of Viruses

Herpes Simplex Virus (HSV)

***Epidemiology*:** Herpes simplex viruses are among the most ubiquitous of human infections. The frequency of HSV infection has been measured by testing various populations for the presence of antibody, as both virus and the immune response are thought to persist after infection for the life of the host. Worldwide, ~90% of people has one or both viruses (Wald and Corey 2007).

***Clinical presentation*:** Infection with the HSV type 1 is mainly in the upper respiratory tract or at the ocular mucosa. As a result of sexual activity HSV type 2 affects genital tract (cervix, vagina, and vulva) and anorectal area. Primary infection is accompanied with general clinical symptoms (pain, lymphadenopathies of regional lymph nodes, and visible lesion). Symptoms start 5–7 days after primary contact, usually as painful papules, then pustules, and ulceration of the skin. Cytological diagnosis of HSV infected cells is usually easy to make, since the morphological changes are hard to miss.

***Cytologic findings*:** Morphologically, changes on cells infected by HSV type 1 or type 2 are pretty much identical (Wolontis 1977; Arduino 2008). Distinction between these two types can be distinguished serologically or by the electron microscopic studies but not morphologically. Cytoplasm of the affected cells becomes dense or opaque with or without degenerative vacuoles. Formations of the diagnostic giant cells are often seen in the smears. They differ from multinucleated foreign-body giant cells by characteristic nuclear changes: hydropic and ballooning degeneration of the chromatin which result in dividing chromatin material (Fig. 1). It loses its



Viral Infections, Cytological Findings, Fig. 2 Herpes simplex virus. Giant cell with condensation of the chromatin reveals ground glass appearance and large inclusion appears in the center of the nuclei, surrounded with clear zone or "halo" (Genital infection, Conventional Pap smear, Papanicolaou ×400)

granularity and starts to clump and adher to inner surface of nuclear membrane leaving amorphous zone in the nuclei center often, a large inclusion appears in the center of the nuclei, surrounded with clear zone or "halo" (Fig. 2). Whole nuclear appearing is commonly referred as glassy, opaque, or ground glass. In majority of cases multinucleation is observed with molding or overlapping of the nuclei (Figs. 3 and 4). Described nuclear pattern along with the inclusions is sufficient for cytological diagnosis.

***Different diagnosis*:** Multinuclear giant cells may be mistaken for the trophoblastic cells or for foreign-body giant cells or reactive multinucleated endocervical cells but latest have preserved chromatin and visible nucleoli.

Herpes Zoster Virus

***Epidemiology*:** Varicella zoster virus (VZV) causes a primary infection known as varicella. During life, it reactivates within a single ganglion to cause a secondary infection known as herpes zoster (HZ). Independent of age, increased risk for reactivation is during disease or therapy of immunocompromised patients. The estimated average overall incidence of HZ is about 3.4–4.82 per 1000 person years which increases to more than 11 per 1000 person years in those aged at least 80 years (Johnson et al. 2015).

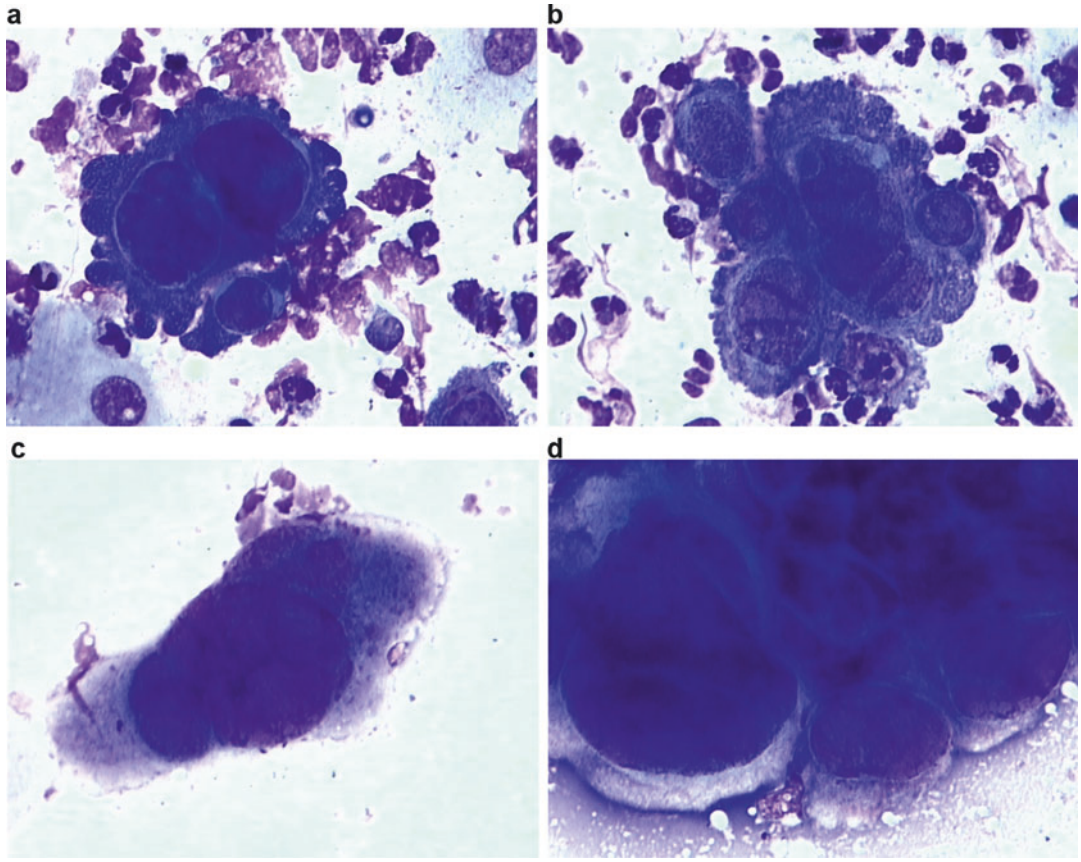
***Clinical presentation*:** Herpes zoster is a disease of nerve tissue. Complications may be dermatological (e.g., secondary bacterial infection), neurological (e.g., long-term pain, segmental paresis, stroke), ophthalmological (e.g., keratitis, iridocyclitis, secondary glaucoma), or visceral (e.g., pneumonia, hepatitis) (Johnson et al. 2015).

***Cytological findings*:** Morphologically, changes are very similar with the one in HSV infection. Slight difference is numerous infected small, single mononucleated parabasal cells with dense cytoplasm and prominent intranuclear inclusions (Fig. 5).

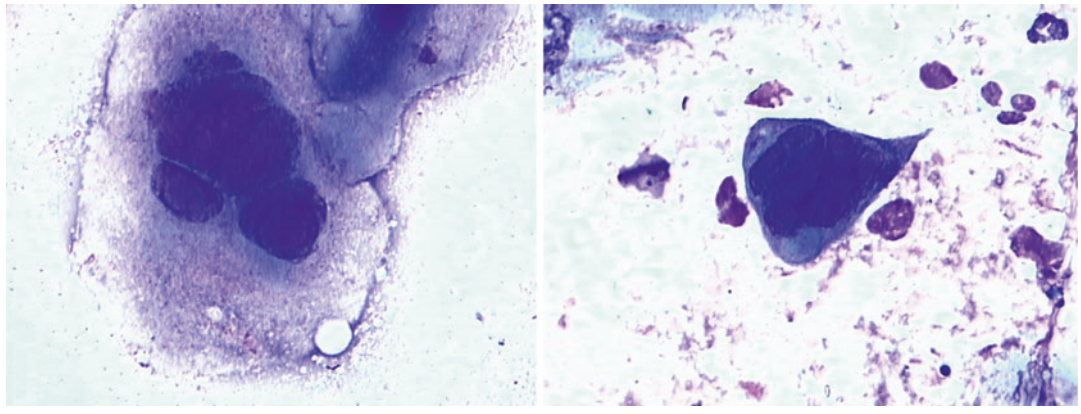
Cytomegalovirus (CMV)

***Epidemiology*:** Cytomegalovirus is classified as a human (beta) herpesvirus 5 (HHV-5) which is transmitted through mucosal contact with infectious tissues, secretions, and excretions (urine, saliva, breastmilk, blood, cervical secretions, and semen) (Lavanchy 2004).

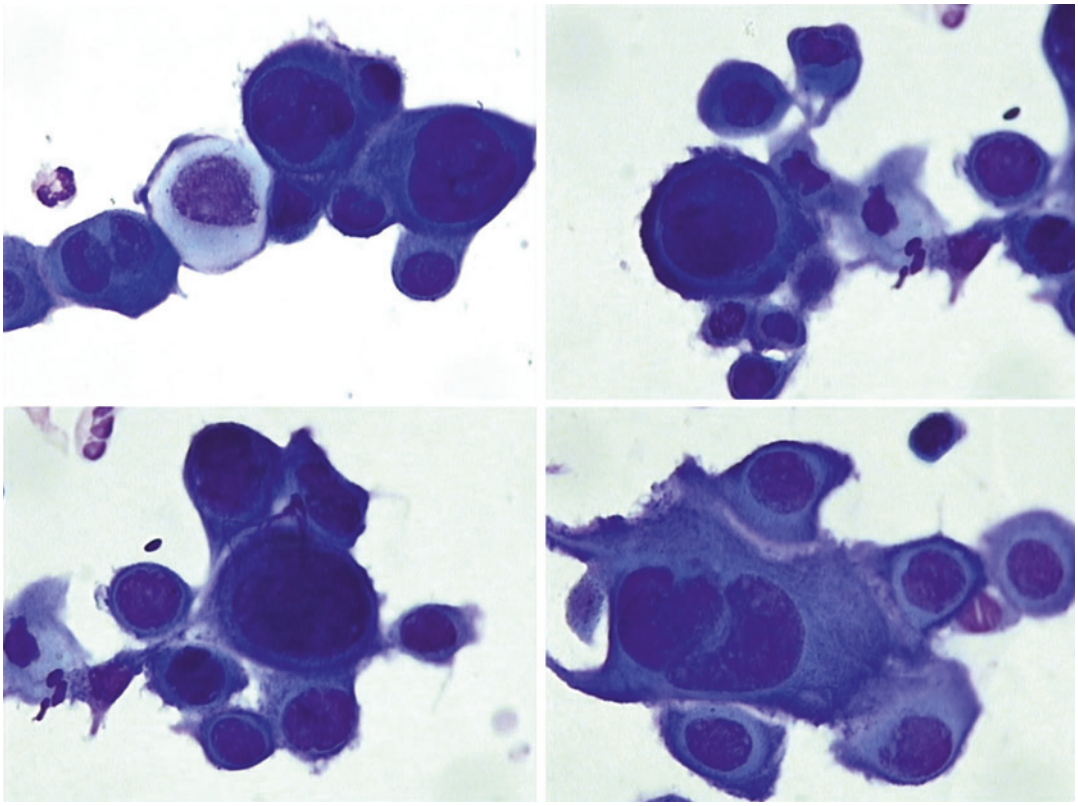
***Clinical presentation*:** CMV infection is common, but in majority of cases are asymptomatic. Sometimes it may cause illness like EBV-infectious mononucleosis syndrome. The most severe form of disease result from fetal and neonatal infection, and it is one of the leading viral causes of birth defects. After primary CMV infection, like other herpesviruses infections, virus produces latent infection in lymphocytes, monocytes, and epithelial cells. If the host becomes immunocompromised CMV infection reactivates and can cause hepatitis, pneumonitis, retinitis, and gastrointestinal tract disorders (Klein et al. 2007; Taylor 2003).



Viral Infections, Cytological Findings, Fig. 3 Herpes simplex virus. Typical giant, multinucleated cell, molding nuclei, large inclusions, and margination of the nuclear membrane (Oropharyngeal swab, May-Grünwald Giemsa ×1000)

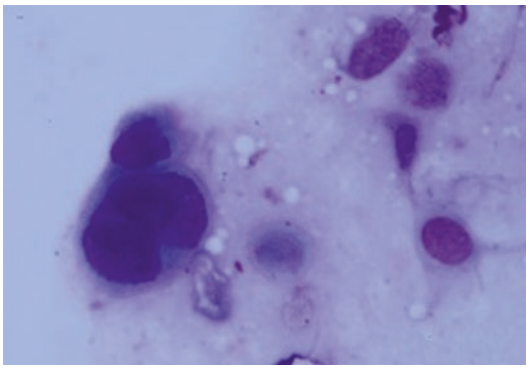


Viral Infections, Cytological Findings, Fig. 4 Herpes simplex virus. Typical giant, multinucleated cell with molding effect (Oro-labial swab, May-Grünwald Giemsa ×1000)



Viral Infections, Cytological Findings, Fig. 5 Herpes zoster virus. Bi- and large multinucleated epithelial cell (Dermal swab, May-Grünwald Giemsa ×1000)

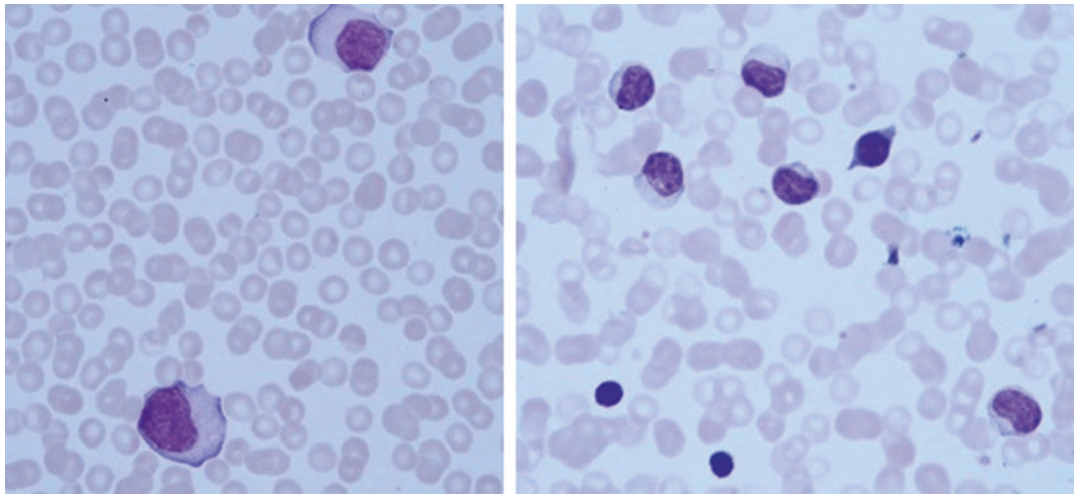
Cytological findings: Small intranuclear inclusion are observed at first, followed by nuclear enlargement with large, single eosinophilic inclusion in the nuclei surrounded by halo which gives the cell characteristic "owl's eye" look (Fig. 6). In spite of great affinity of endocervical cell for CMV, cervical cytology is not a good tool for detecting this infection. Reactive lymphocytes in peripheral blood smear in acute manifestations of CMV infection are enlarged with slightly irregular nuclei, loose chromatin, and abundant cytoplasm intensively basophilic on the cellular edges (Fig. 7).



Viral Infections, Cytological Findings, Fig. 6 Cytomegalovirus. Binuclear epithelial cell with large intranuclear inclusions. In the background are few other degenerative cells (Oropharyngeal swab, May-Grünwald Giemsa ×1000)

Human Papilloma Virus (HPV)

Epidemiology: These viruses belong to the family of papovaviride which include papilloma and

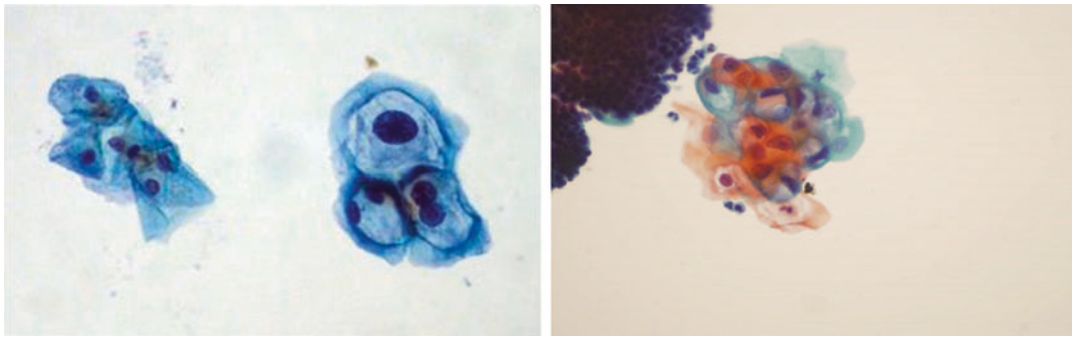


Viral Infections, Cytological Findings, Fig. 7 Cytomegalovirus. Reactive lymphocytes in peripheral blood smear are enlarged with slightly irregular nuclei, loose chromatin, and abundant cytoplasm intensively basophilic on the cellular edges (Peripheral blood smear, May-Grünwald Giemsa ×1000)

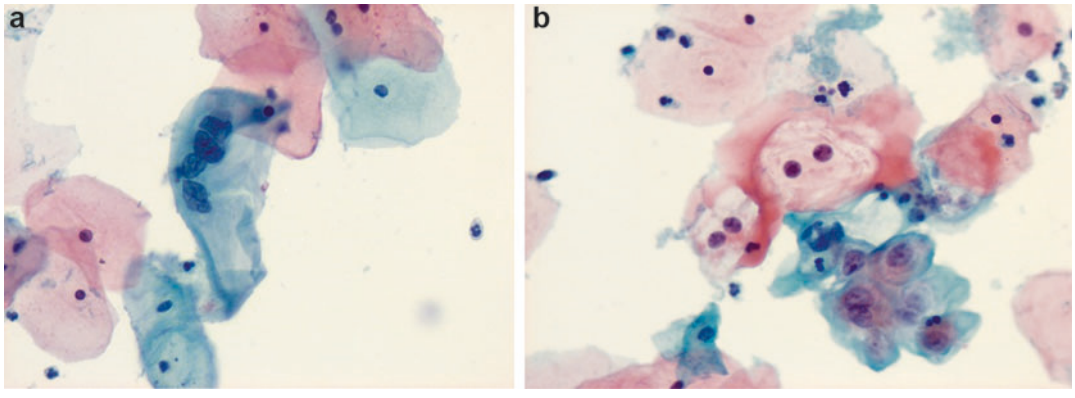
polyomaviruses or viruses with double-stranded DNA. They have tropism for the skin or mucosa and are causes of various lesions on humans and/or animals.

Clinical presentation: The vast majority of those viruses infect skin or mucosa surfaces and cause a warty growth (Richart 1969; zur Hausen 1991). Due to molecular diagnostic techniques and methods, over 120 different types of HPV have been identified so far (Kjaer 2002). These different HPV types tend to be site-specific. That means that certain HPV types and specific lesion are associated. For example, types 1, 2, 3, 4, 10, or 28 are accompanied with common warts; types 6 and 11 are mostly associated with anogenital condyloma; and types 16, 18, 31, 33, etc., are found in almost 97% of high-grade squamous intraepithelial lesion (HG-SIL) and therefore are called high risk or oncogenic HPV types (hr HPV) (Richart 1969; zur Hausen 1991; Walboomers 1999; Bosh 2002).

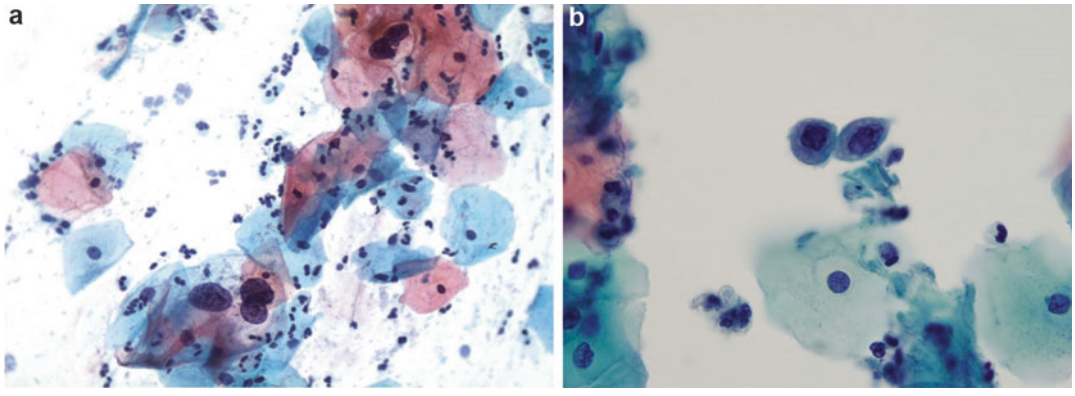
Cytological findings: HPV affects simultaneously cytoplasm as well as nucleus of the infected cell. One of the earliest cellular changes is enlargement of the cytoplasm of intermediate and/or parabasal cells. Dyskeratosis and koilocytes are classic cytological findings and can be considered as pathognomonic sign for HPV infection. A koilocyte is an infected squamous cell – most commonly intermediate type, with characteristic perinuclear halo. Cytoplasm of these cells shows peripheral condensation leaving a wide, empty space around the nucleus with sharp peripheral margins (Fig. 8). The nuclei are enlarged and often bi- or multinucleated and variations of the cells and nuclei dimension and shape are present (Fig. 9). Chromatin is often coarsely granular showing an apparent hyperchromasia of the nuclei (Fig. 10) and sometimes they can be picnotic. Nuclei are usually located eccentrically, very seldom centrally. Nucleoli can be inconspicuous in these cells and nuclear membrane may be wavy, partly molding resulting in "raisin"-like appearance (Fig. 11). Presence of plaque formation or clusters of rounded squamous cells with dense, keratinized cytoplasm and hyperchromasia and polymorphism are often seen (Fig. 12a). Parakeratosis (Fig. 12b) and hyperkeratosis are not suggestive only for HPV infection but such samples must be carefully examined since HPV infection can reveal abnormal keratinization. Others, so-called nonclassic HPV signs like spindle cells, cytomegaly, atypical parakeratosis, or atypical



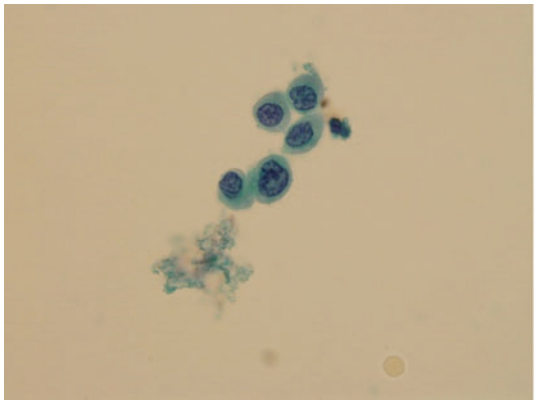
Viral Infections, Cytological Findings, Fig. 8 Human papilloma virus. Koilocyte – a squamous cell whose cytoplasm shows peripheral condensation leaving a wide, empty space around the nucleus with sharp peripheral margins. Notice the binucleation of the cell (Liquid based cytology of cervical sample, Papanicolaou ×400)

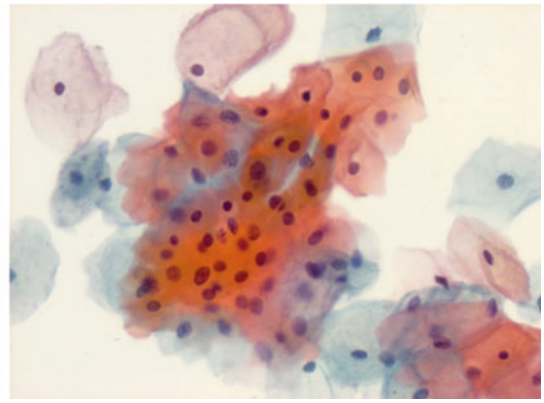


Viral Infections, Cytological Findings, Fig. 9 Human papilloma virus. (**a**) Multinucleated cell with marked variation of the nuclei dimension and shape; (**b**) Binucleated koilocytes in the intermediate squamous cells (Conventional Pap smear, Papanicolaou ×400)

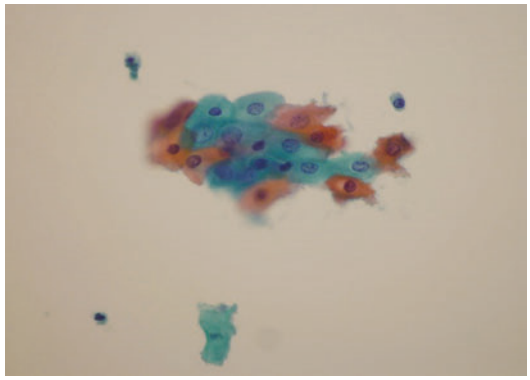


Viral Infections, Cytological Findings, Fig. 10 Human papilloma virus. (**a**) Binucleated koilocytes and multinucleated cells with hypechromatic, enlarged nuclei (Conventional Pap smear, Papanicolaou ×400); (**b**) Nuclei of infected cells are eccentrically situated and chromatin is coarsely granular showing an apparent hyperchromasia (Liquid based cytology of cervical sample, Papanicolaou ×400)





Viral Infections, Cytological Findings, Fig. 11 Human papilloma virus. (**a**) Wavy nuclear membrane, partly molding resulting in "raisin"-like appearance; (**b**) A plaque formation or clusters of rounded squamous cells with dense, keratinized cytoplasm, hyperchromasia, and polymorphism (Conventional Pap smear, Papanicolaou ×400)



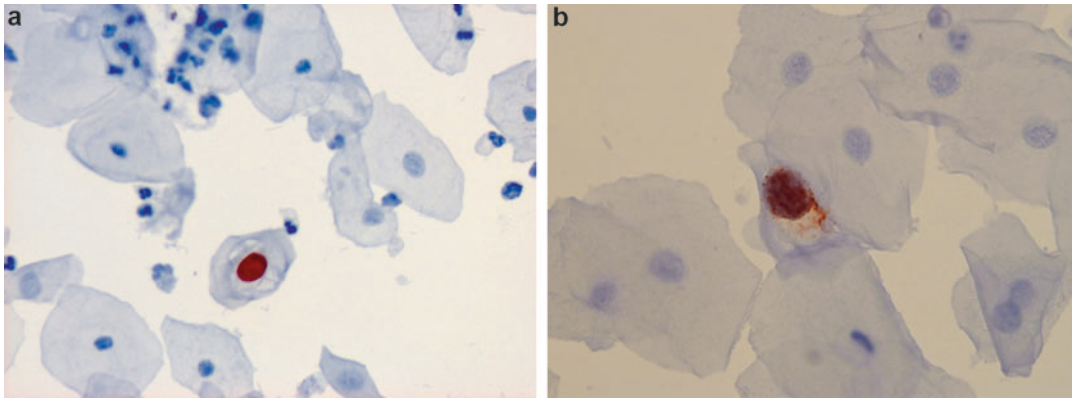
Viral Infections, Cytological Findings, Fig. 12 Human papilloma virus. Abnormal keratization – parakeratosis (Conventional Pap smear, Papanicolaou ×400)

metaplasia, can be suggestive of HPV infection in cervical smears.

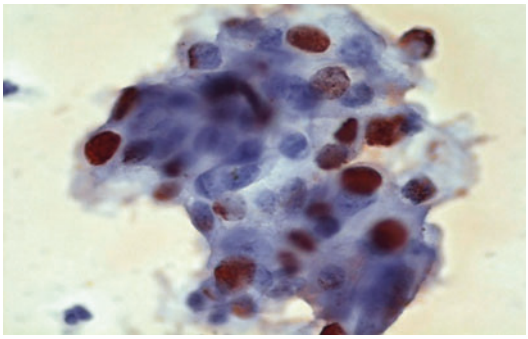
Cytology as a method of screening is not intended to be diagnostic for recognition of the HPV infections, but it can be an excellent tool in distinguishing unaffected women from those who may have disease, so ideally its result requires a confirmation by molecular methods of diagnosis.

But, for understanding morphologic features of HPV infection we must explain HPV life cycle. HPV genome consists of three gene groups: control region genes (LCR), structural genes (L1 and L2), and early E-genes which are responsible for gene expression control and for interaction with host cell proteins. After the epithelial trauma HPV enters the basal cells and its genome is transferred to the host cell nucleus. Virus starts to replicate using replication mechanism of the infected cells. Early genes (E6, E7) are modifying surroundings of infected cells so their differentiation into keratocytes can begin. At the same time E5 protein participates in raising the level of different mitogenic factors necessary for basal cell proliferation or to start one. Because of the E1 and E2 proteins, that proliferation is under control so basal cells can differentiate to keratinocytes. Those proteins control the HPV genome replication so E4 protein and structural genes (L1 and L2) can do their part in formatting a thousand copies of the virions (von Knebel Doeberlitz 2002).

Data showed that by molecular methods, biologic potentiality of the lesion can be



Viral Infections, Cytological Findings, Fig. 13 Human papilloma virus. Immunocytochemistry. (**a**) Positive HPV L1 staining for virions within nucleus, (**b**) Positive nucleus of the koilocyte (Conventional Pap smear, Cytoimmun Diagnostic GbmH, Pirmanses, Germany, High pH ×400)



Viral Infections, Cytological Findings, Fig. 14 Human papilloma virus. Immunocytochemistry. HPV L1 positive group of LSIL cells (Conventional Pap smear, Cytoimmun Diagnostic GbmH, Pirmanses, Germany, High pH ×400)

estimated and that an expression pattern of some biomarkers could be useful in planning the clinical management, so unnecessary treatments and patients' distress can be avoided (Tornesello 2013).

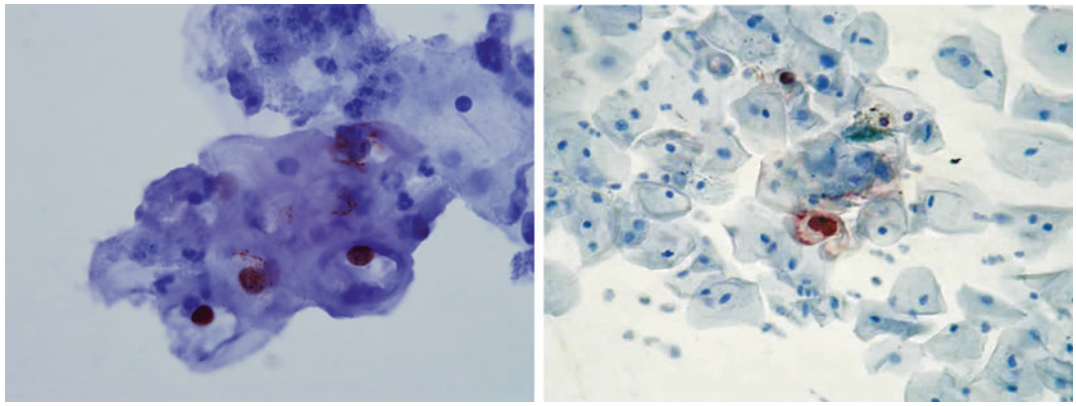
HPV L1

Viral capsid protein L1 (HPV L1) is expressed in early phase of the infection (Figs. 13, 14, and 15) and disappears in the later phases of carcinogenesis, suggesting its potential role in predicting the outcome of cervical intraepithelial lesion (Griesser 2004; Rauber 2008; Mehlhorn 2013).

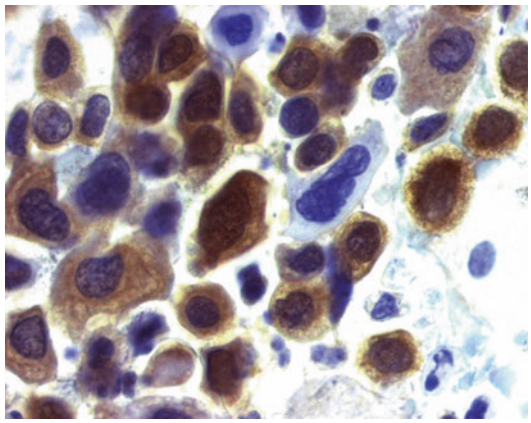
p16INK4A

Regular cellular protein p16INK4a (p16) usually gets overexpressed when specific viral oncogenes start to interfere with the host's cell-cycle regulation system (Bergeron 2015). Expression of p16 underlies a negative feedback control through pRB (retinoblastoma oncoprotein). Inhibited function of pRb should results in overexpression of p16 levels, making it a specific marker for cells with the expression of HPV oncogenes. The value of expression has already been demonstrated as a surrogate marker for the oncogenic activities of HPV in the cells of the cervical epithelium (Klaes 2001; von Knebel Doeberlitz 2002; Wentzensen 2007) as well as a tool that improves intraoberver agreement in the diagnosis of cervical intraepithelial lesions (Klaes 2002; Horn 2008).

The inclusion of immunocytostaining has proved the use of p16INK4a both as a marker for the presence of HPV infection and as a marker of premalignant epithelial changes. Positive staining



Viral Infections, Cytological Findings, Fig. 15 Human papilloma virus. Immunocytochemistry. HPV L1 positive group of koilocytes (Conventional Pap smear, Cytoimmun Diagnostic GbmH, Pirmanses, Germany, High pH ×400)



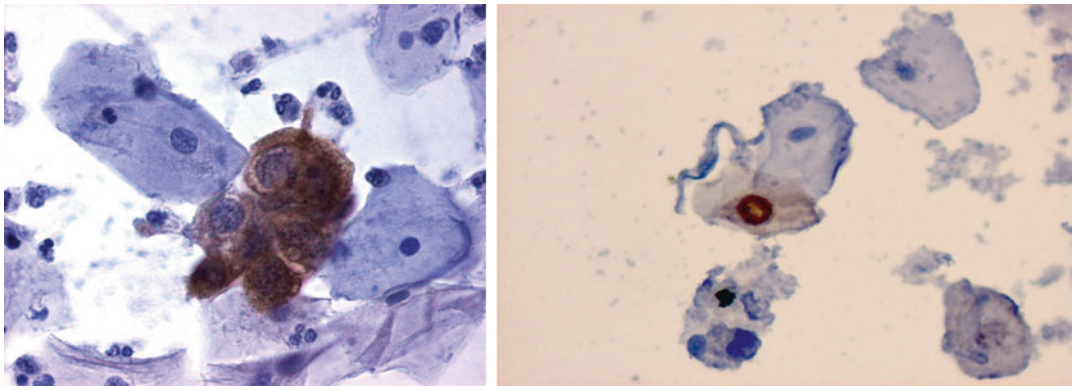
Viral Infections, Cytological Findings, Fig. 16 Human papilloma virus. Immunocytochemistry. P16INK4a positively stained cytoplasm with/without *brownish* stained nuclei in HSIL lesion (Conventional Pap smear, CinTec by MTM Laboratories AG, Heidelberg, High pH ×400)

reaction showed brown colored nuclei, particular or accompanied with brownish cytoplasm of the infected cells (Figs. 16, 17, and 18). That confirms the integration of the virus genome into the host cell genome and indirectly verifies the presence of virus. This demonstrates the genetic instability of the cell and impossibility of improvement in test results, inferring the evaluation of p16INK4a positivity to be not only a valuable diagnostic tool to differentiate cytological abnormalities from normal findings, but also is a very informative prognostic indicator of the need to monitor p16 positive patients due to the possible development of more advanced premalignant lesions and even carcinoma in such patients.

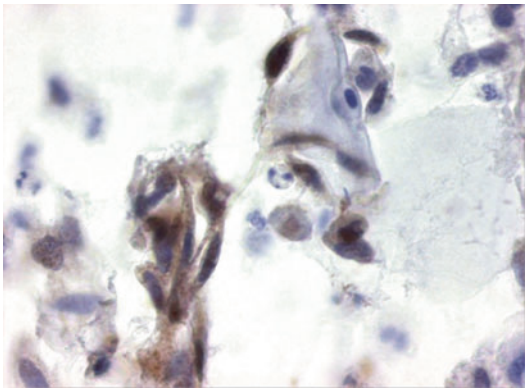
The individual use of HPV L1 staining is questionable because of its low sensitivity, but the combination of the two markers accompanied by the cytological follow-up of lesions is useful in the identification and monitoring of the patients at risk of lesion progression.

Dual Staining p16/Ki67

High proportion of the cervical intraepithelial lesions shows proliferative activity as well. The expression of both markers – the proliferation marker Ki-67 and p16INK4a – within the same cell should mutually exclude each other under physiological conditions. This means that the detection of cervical epithelial cells which are simultaneously expressing both p16 and Ki67 (Figs. 19 and 20) may be used as an indicator for the deregulation of the cell-cycle control in the



Viral Infections, Cytological Findings, Fig. 17 Human papilloma virus. Immunocytochemistry. Positive CinTec reaction in HSIL lesion (Conventional Pap smear, CinTec by MTM Laboratories AG, Heidelberg, High pH ×400)



Viral Infections, Cytological Findings, Fig. 18 Human papilloma virus. Immunocytochemistry. p16INK4a positive squamous malignant cells (Conventional Pap smear, CinTec by MTM Laboratories AG, Heidelberg, High pH ×400)

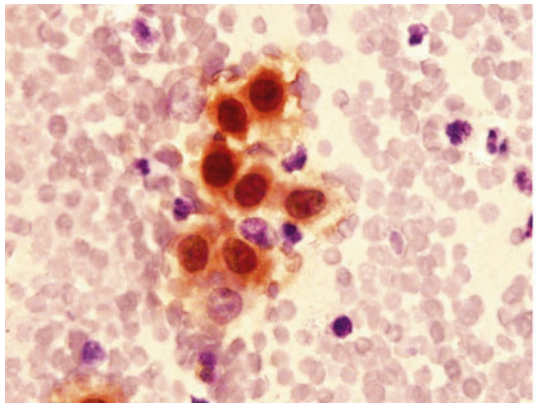
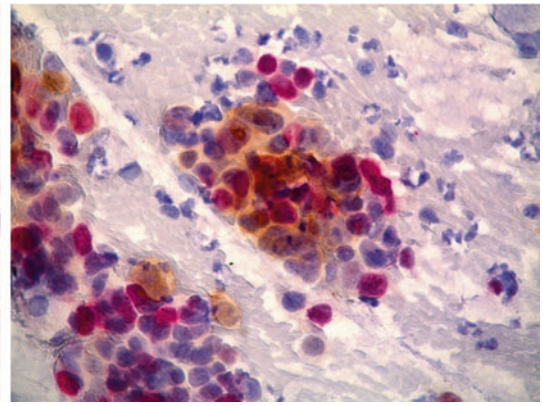
dysplastic epithelial cells and a pointer of a transformed cell status (Schmidt 2011; Petry 2011; Wentzensen 2012).

***Differential diagnosis*:** Sometimes koilocytes can be mistaken for inflammatory reactions on cells caused by trichomoniasis or fungal infection. Abnormal keratization and cells with dense, keratinized cytoplasm and hyperchromatic nuclei resemble the cells of well-differentiated keratinizing squamous carcinoma.

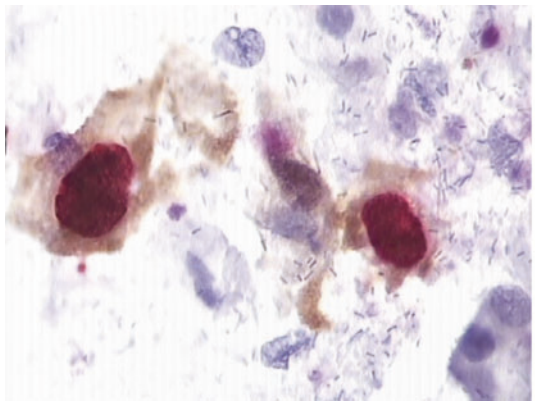
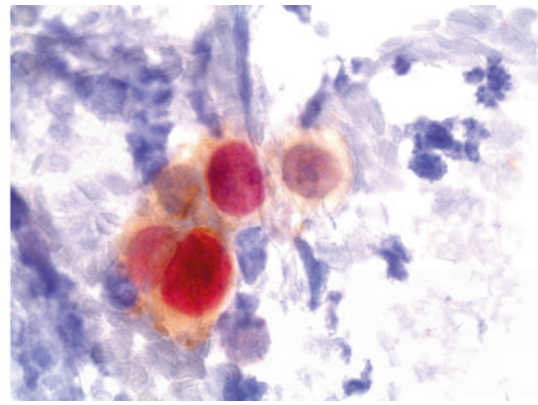
Polyomavirus (PV)

***Epidemiology*:** Polyomaviruses (PV) are small, nonenveloped, double-stranded, 40–44 nm DNA viruses. Three species, BK virus (BKV), JC virus (JCV), and Simian virus 40 (SV40), are associated with disease in humans. They are ubiquitous in nature. Approximately 60–80% of adults are serologically positive to PV. Primary infection occurs in early childhood trough oral or respiratory routes. In immunocompetent individuals, after the primary infection, PV commonly remains latent within several different tissues, especially in transitional cells and renal tubular epithelial cells (Trofe et al. 2004; Nickeleit et al. 1999; Maia et al. 2011; Nickeleit et al. 2002).

***Clinical presentation*:** The reactivation of latent PV occurs during immunocompromised conditions, such as pregnancy, diabetes mellitus, cancers, HIV infection, and organ transplantation. There are different diseases caused by polyomaviruses, depending on the viral species (BKV, JCV, SV40) and the underlying reason for immunosuppression. JCV may cause progressive multifocal leukoencephalopathy in patients with

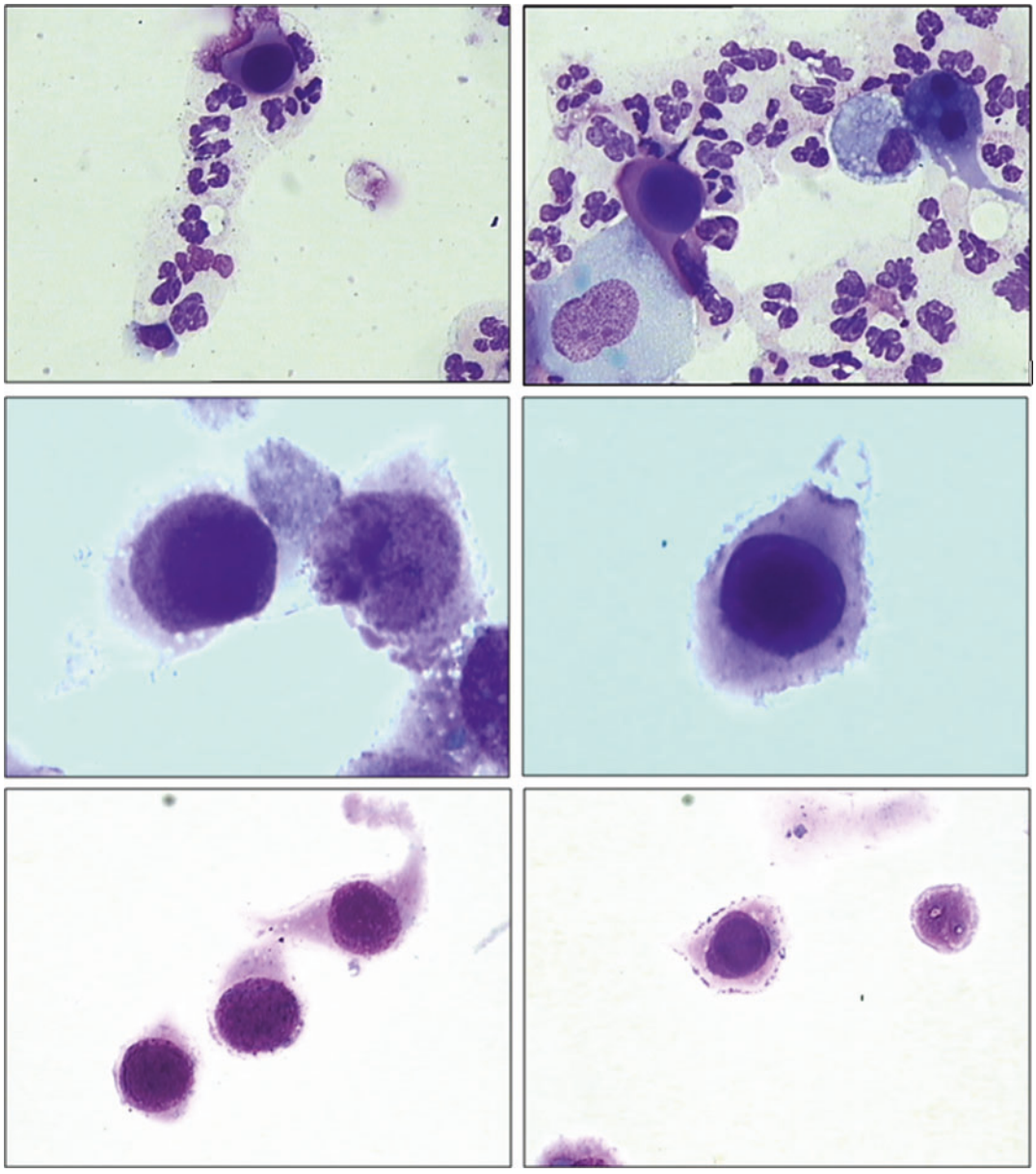
Viral Infections, Cytological Findings, Fig. 19 Human papilloma virus. Immunocytochemistry. Dual staining p16/Ki67 positive cluster of cells (Conventional Pap smear, CinTec by MTM Laboratories AG, Heidelberg, High pH ×400)

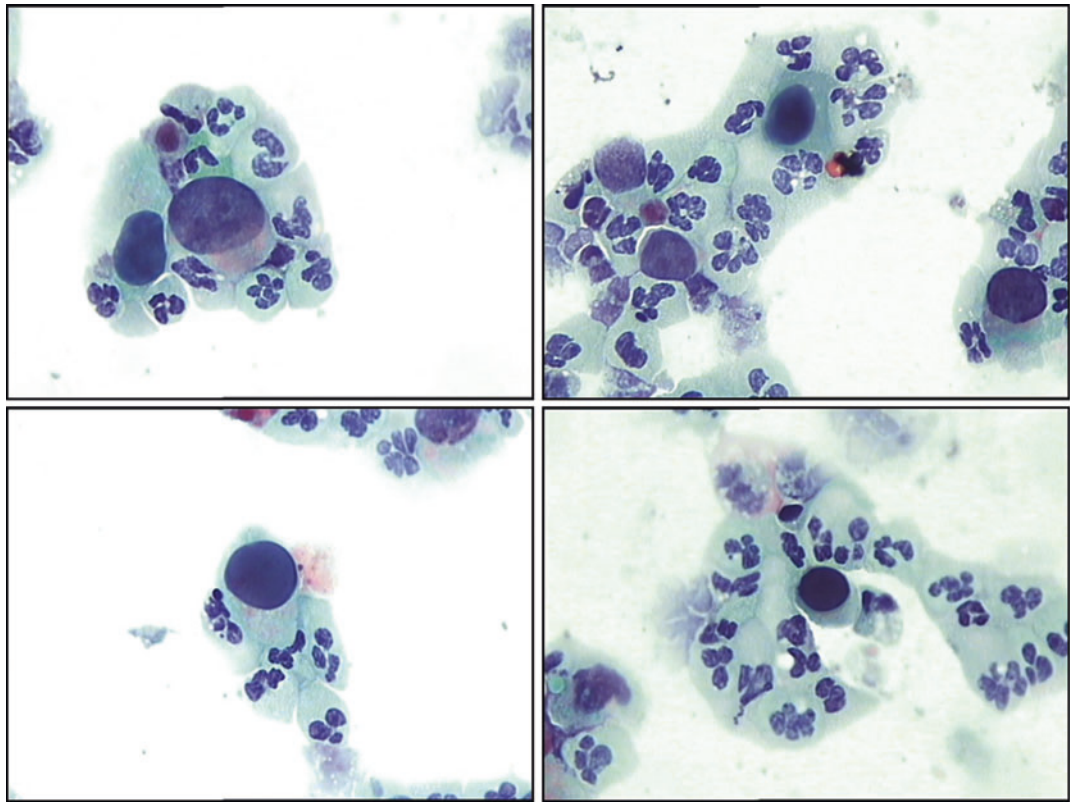
Viral Infections, Cytological Findings, Fig. 20 Human papilloma virus. Immunocytochemistry. *Red* nuclei and *brownish* cytoplasm for positive p16/Ki67 dual staining in LBC sample (Liquid based cytology of cervical sample, CinTec by MTM Laboratories AG, Heidelberg, High pH ×400)

HIV infection. In bone marrow transplant recipients, the BKV reactivation is associated with a hemorrhagic cystitis. BK virus nephropathy (BKN) is the most important infectious complication in kidney transplant recipients. It affects approximately 1–10% of patients resulting in chronic allograft dysfunction or even loss in up to 1–5% of kidney transplant recipients (Bohl and Brennan 2007; Trofe et al. 2004; Nickeleit et al. 2002; Vidas et al. 2010; Bogdanovic et al. 2004; Singh et al. 2006; Gracin et al. 2010; Kovačević-Vojtusek et al. 2010).

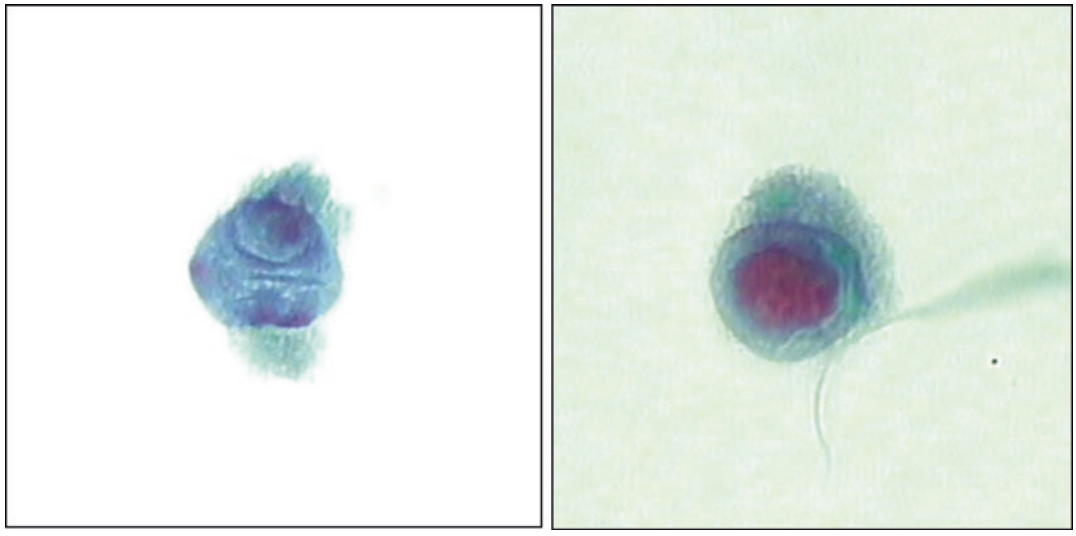
***Cytological findings*:** A morphologic evidence of BKV activation and replication is the detection of infected transitional cells with intranuclear inclusion bodies, known as "decoy cells" in the



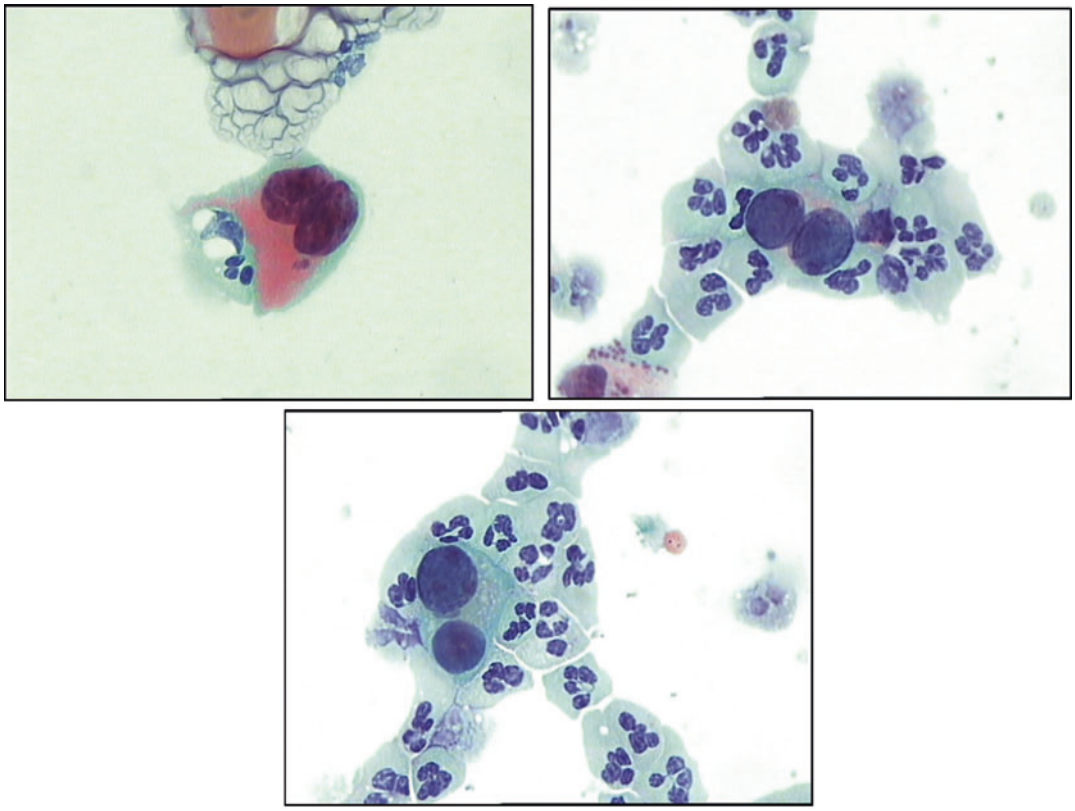
Viral Infections, Cytological Findings, Fig. 21 Polyomavirus. Classical decoy cells characterized by single, large, basophilic, homogenous, ground-glass like intranuclear inclusions and a thin, condensed rim of chromatin at the periphery of the nuclei (Urine cytology, May-Grünwald-Giemsa staining, ×1000)



Viral Infections, Cytological Findings, Fig. 22 Polyomavirus. Type 1 decoy cells with nuclear enlargement and large, homogenous intranuclear viral inclusion bodies (Urine cytology, Papanicolaou ×1000)



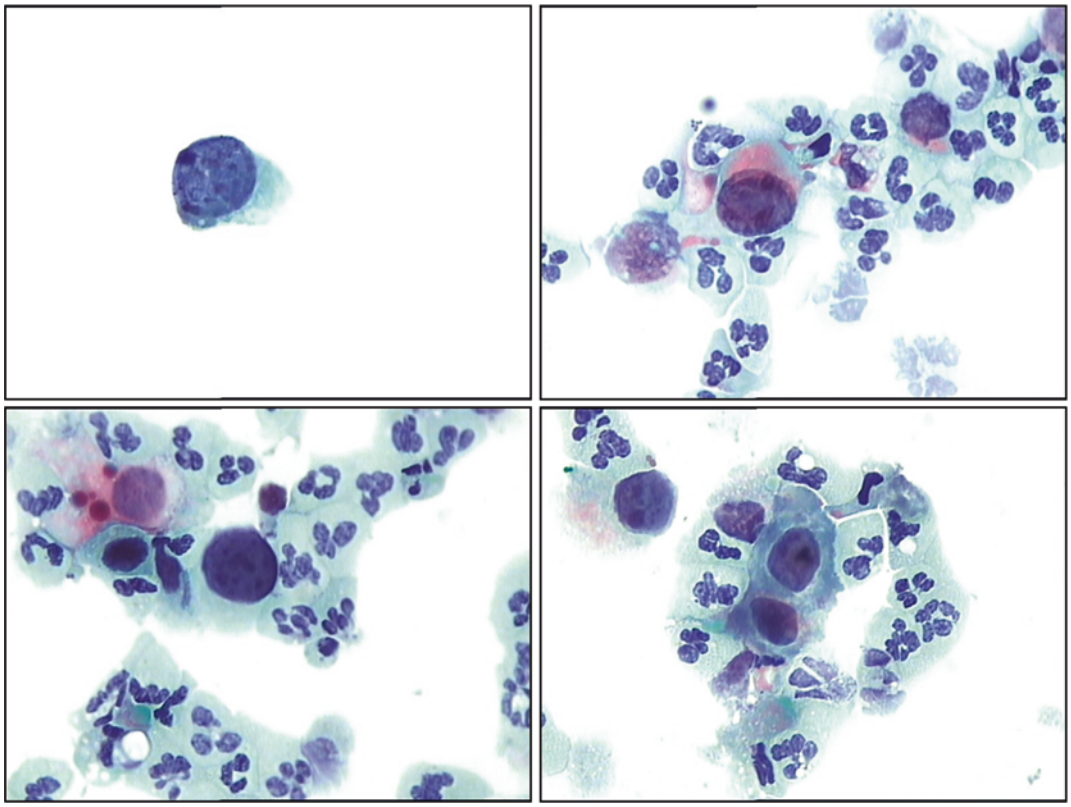
Viral Infections, Cytological Findings, Fig. 23 Polyomavirus. Type 2, cytomegalovirus (CMV)-like decoy cells with granular intranuclear inclusions surrounded by a clear, irregular, incomplete halo (Urine cytology, Papanicolaou ×1000)



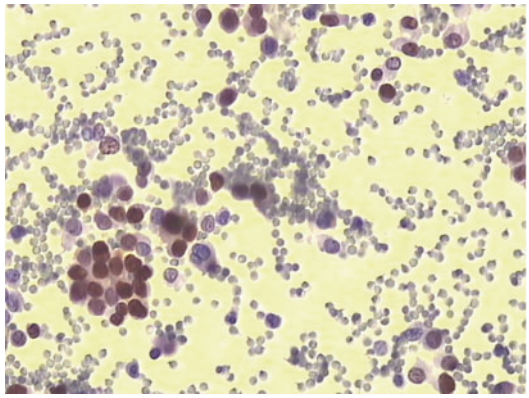
Viral Infections, Cytological Findings, Fig. 24 Polyomavirus. Type 3 decoy cells with a finely granular chromatin and multinucleation (Urine cytology, Papanicolaou ×1000)

urine. Four different phenotypes of decoy cells are described. The most frequent, classical decoy cells show nuclear enlargement with single, large, basophilic, homogenous, ground-glass like intranuclear inclusions (type 1) and a thin rim of condensed chromatin (Figs. 21 and 22). Type 2, cytomegalovirus (CMV)-like decoy cells contain eosinophilic, granular intranuclear inclusions surrounded by a clear, irregular, usually incomplete halo (Fig. 23). Type 3 decoy cells are multinucleated with a finely granular chromatin (Fig. 24). A vesicular variant of decoy cells (type 4) shows a coarsely granular and clumped chromatin, sometimes with visible nucleoli (Fig. 25) (Singh et al. 2006; Nickeleit et al. 2002; Rosenthal et al. 2016).

***Differential diagnosis*:** High-grade malignant urothelial cells should be considered in the differential diagnosis of decoy cells, especially type 3 and type 4 of decoy cells. Because of their nuclear enlargement and hyperchromasia, decoy cells can be misinterpreted as malignant cells. In contrast to most malignant cells, which commonly show irregular nuclear membranes, nuclear membranes of decoy cells are very smooth and regular in shape. Unlike malignant cells, which usually tend to form cell clusters, decoy cells are commonly found as single cells (Singh et al. 2006; Rosenthal et al. 2016; Khaled 2004). Decoy cells are immunocytochemical positive for BK virus (Fig. 26).



Viral Infections, Cytological Findings, Fig. 25 Polyomavirus. Type 4 decoy cells with a coarsely granular and clumped chromatin and visible nucleoli (Urine cytology, Papanicolaou ×1000)

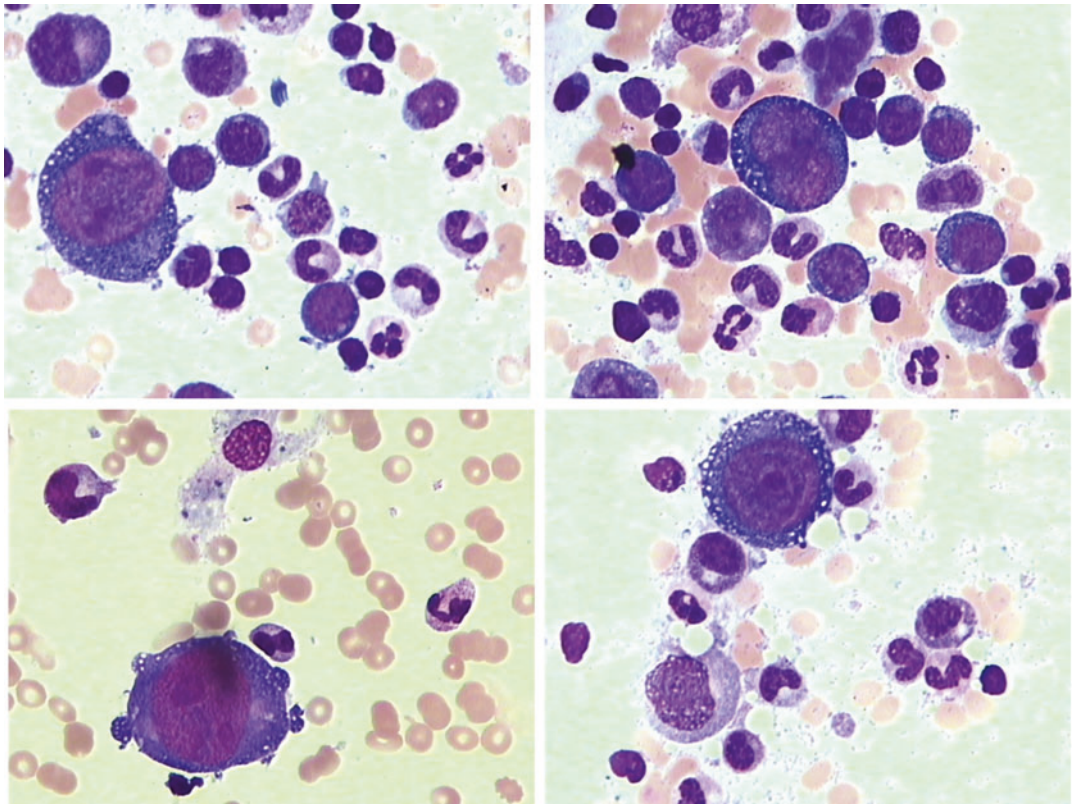


Viral Infections, Cytological Findings, Fig. 26 Polyomavirus. Immunocytochemistry. Positive immunocytochemical staining for BK virus in decoy cells (Urine cytology, immunoperoxidase ×400)

Parvovirus B19

***Epidemiology*:** Human parvovirus B19 is a 20–25 nm DNA virus belonging to the *Parvoviridae* family. It is usually transmitted by respiratory secretion and often produces mild erythematous disease in children (Heymann 2004). This virus shows tropism to human erythroid precursor cells which it selectively infects and destroys them at the colony unit stage. The globoside known as blood group P antigen is the receptor by which the virus enters the cell (Young and Brown 2004).

***Clinical presentation*:** Severe forms of illness can rarely occur as transient aplastic crisis in patients with underlying hemolytic diseases like



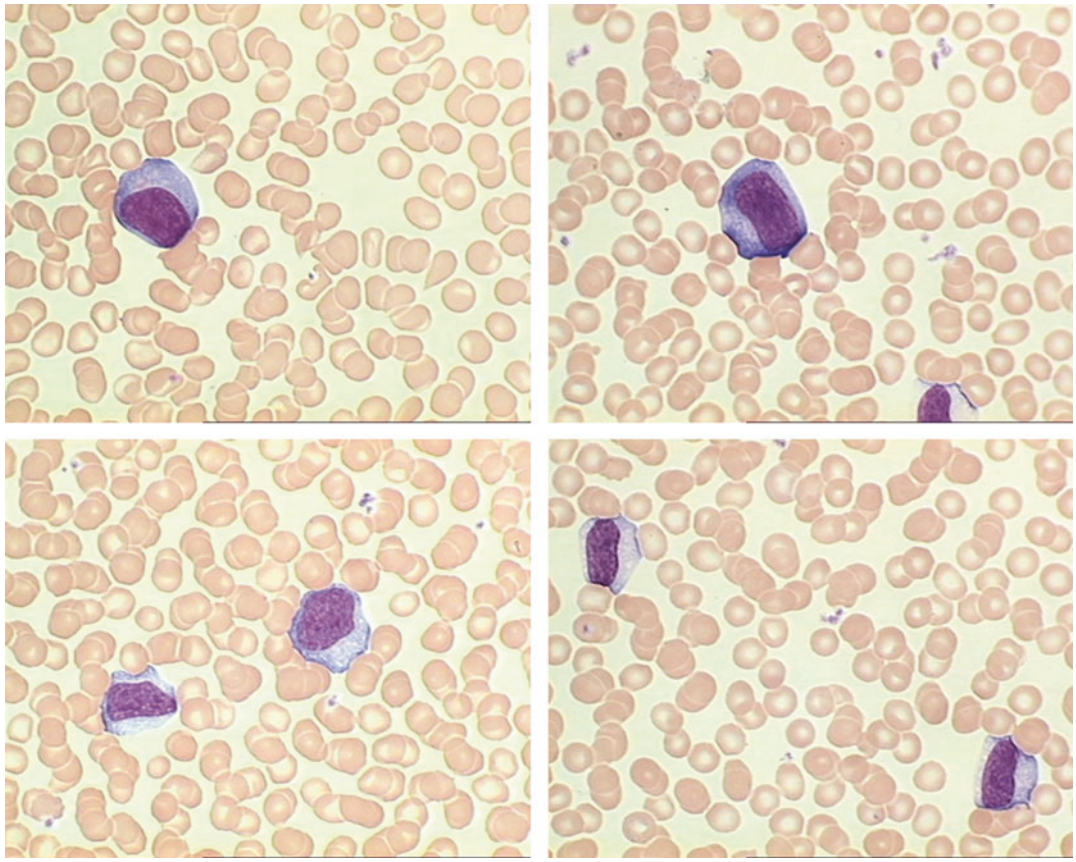
Viral Infections, Cytological Findings, Fig. 27 Parvovirus B19 Virus. The cytopathic effect of parvovirus B19. Absence of erythroblasts in the otherwise normal bone marrow with the emergence of very large cells – giant proerythroblast with abundant deeply basophilic cytoplasm, large nuclei with delicate chromatin, and prominent nucleoli (Bone marrow smear, May-Grünwald Giemsa ×1000)

hereditary spherocytosis, sickle cell disease, thalassemia, etc. In immunocompromised states, because of lack of protective antibodies, persistent parvovirus B19 infection may develop. The clinical manifestation is pure red cell aplasia with serious anemia associated with absence of reticulocytes (Young and Brown 2004; Sawada et al. 2008).

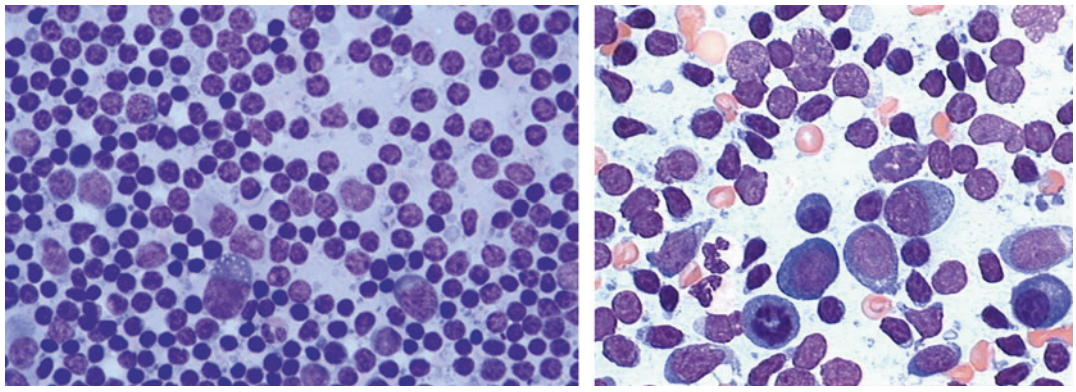
***Cytological findings*:** The characteristic morphologic feature of bone marrow smears is near disappearance of erythroid precursors and occasionally the scarce giant proerythroblasts as a sign of erythropoesis recovery (Fig. 27).

Epstein-Barr Virus (EBV)

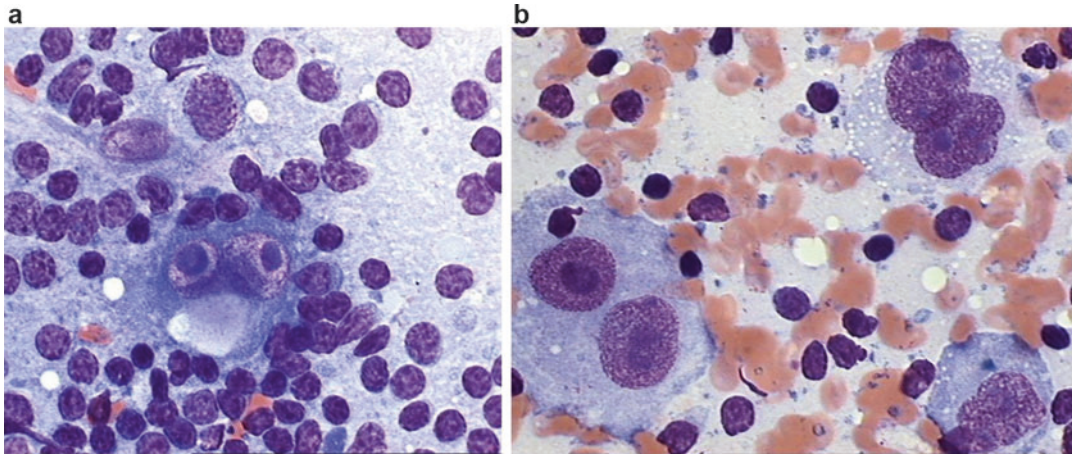
***Epidemiology*:** Epstein-Barr virus (EBV) is 100–200 nm large, enveloped virus belonging to *Herpesviridae* family. It is classified as a human (gamma) herpesvirus 4 (HHV-4) with double-stranded DNA genome, 172 kb in length. It spreads by oropharyngeal route through saliva and very rarely by blood. It is highly prevalent in



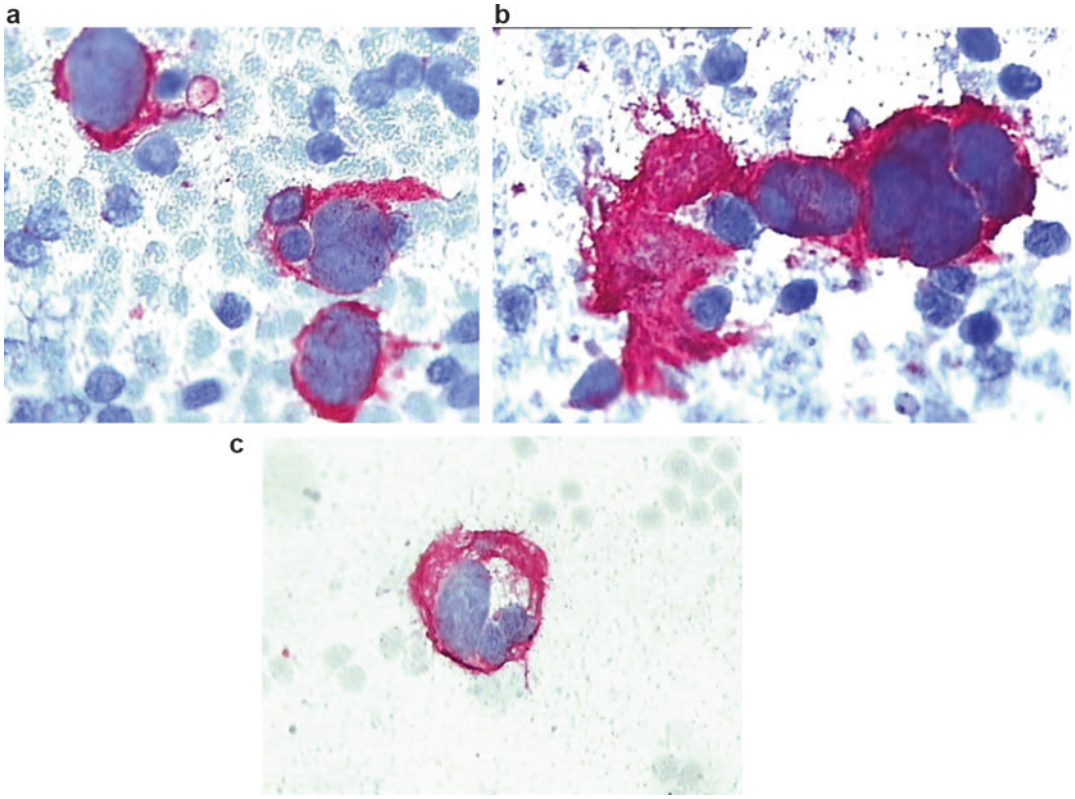
Viral Infections, Cytological Findings, Fig. 28 Infectious mononucleosis. Reactive lymphocytes in peripheral blood smear (Peripheral blood smear, May-Grünwald Giemsa ×1000)



Viral Infections, Cytological Findings, Fig. 29 Infectious mononucleosis syndrome. Very celular sample with numerous transformed lymphatic cells including centrocytes, centroblasts, immunoblasts, proplasma, and plasma cells (Lymph node aspirate smear, May-Grünwald Giemsa ×1000)



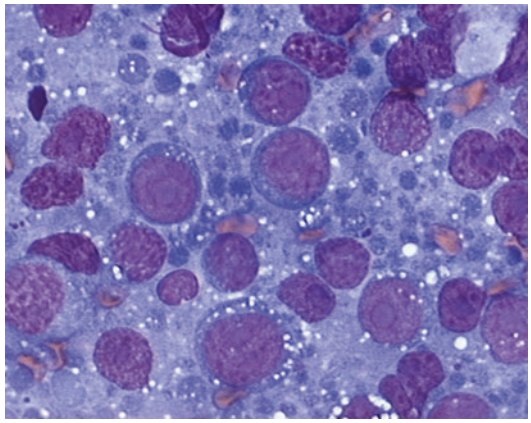
Viral Infections, Cytological Findings, Fig. 30 Hodgkin lymphoma. (**a**, **b**) Diagnostic Reed-Sternberg and Hodgkin cells with very large nuclei, distinct dark nucleoli, and plentiful *grayish* cytoplasm. In the background are small lymphocytes (Lymph node aspirate smear, May-Grünwald Giemsa ×1000)



Viral Infections, Cytological Findings, Fig. 31 Hodgkin lymphoma. Immunocytochemistry. (**a**, **b**) CD30 and (**c**) CD15 positive Reed-Sternberg cells (Lymph node aspirate smears, LSAB ×1000)

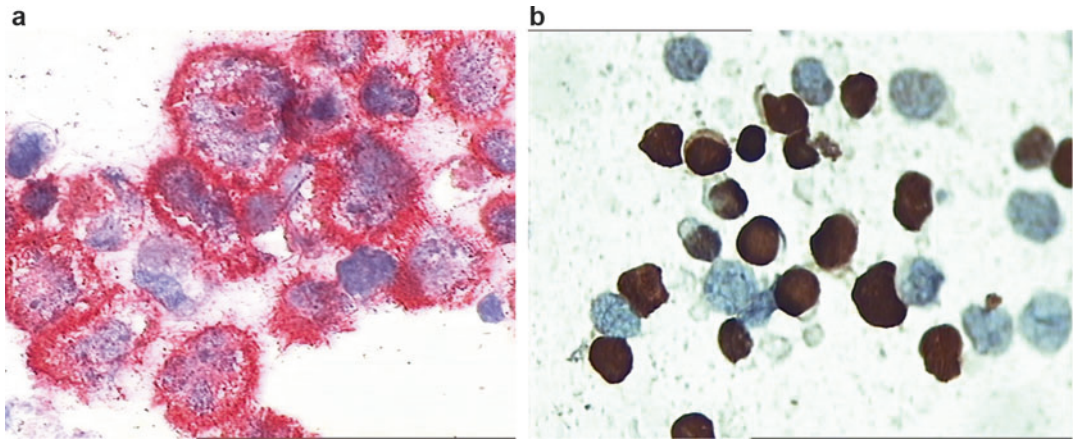
general population, and primary infection often occurs in early childhood as unapparent disease.

***Clinical presentation*:** In adolescents or young adults infection often produces mild to severe acute viral illness called infectious mononucleosis (IM). It is manifested by fever, sore throat, lymphadenopathy, and splenomegaly. EBV as other herpesviruses has a distinctive, biphasic lifecycle. When orally transmitted, primarily infects mucous epithelial cells and B-lymphocytes within oropharyngeal lymphatic tissue. The infection stimulates proliferation and transformation of B-lymphocytes and creates cellular and humoral immunologic EBV response. After that, EBV modulates host immunity; the viral genome is silenced to keep the balance between viral activity and immunosurveillance. The result is latent EBV infection persisting for lifetime in memory B-cells. During the time, this balance can be disturbed by different factors like acquired immune defects, immunosuppresive drugs, stress, or aging process. In that cases, reactivation of infection or active virus replication occurs (Grinde 2013).

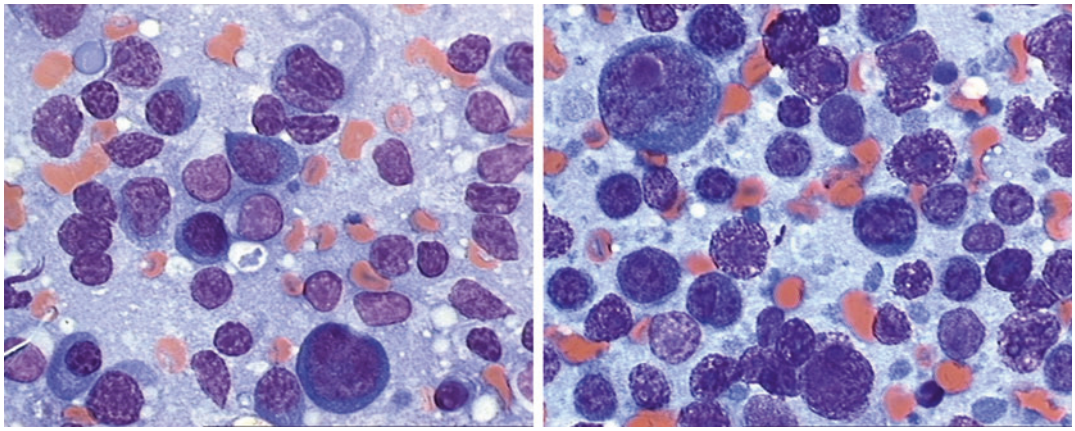


Viral Infections, Cytological Findings, Fig. 32 Non-Hodgkin lymphoma – Burkitt lymphoma. Monomorphic proliferation of large lymphoid cells with delicate chromatin, few small nucleoli, and narrow rim of vacuolated cytoplasm (Lymph node aspirate smear, May-Grünwald Giemsa ×1000)

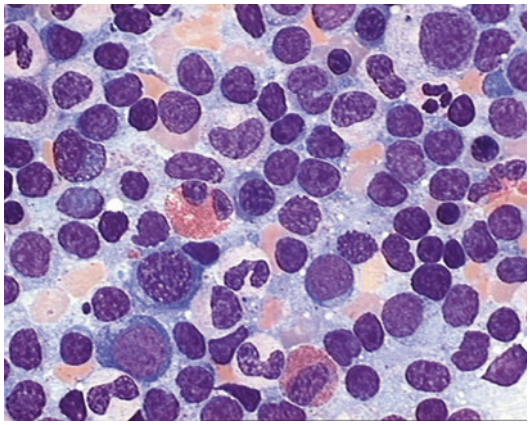
***Cytological findings*:** Infectious mononucleosis presents with characteristic laboratory findings of lymphocytosis and 10% or more reactive lymphocytes (Fig. 28) (Heymann 2004). In the regional swollen lymph nodes there is reactive lymphoid hyperplasia as host response to viral



Viral Infections, Cytological Findings, Fig. 33 Non-Hodgkin lymphoma – Burkitt lymphoma. Immunocytochemistry. (**a**) Cells strongly immunostained for CD20 (Lymph node aspirate smears, LSAB ×1000) and (**b**) Ki67 (Lymph node aspirate smears, HRP ×1000)

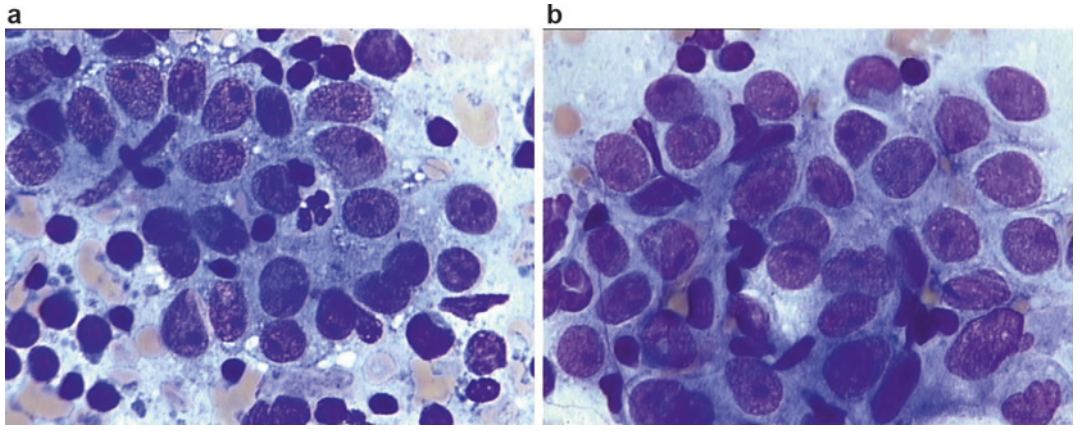


Viral Infections, Cytological Findings, Fig. 34 Non-Hodgkin lymphoma – Peripheral T-cell lymphoma. Small, medium-sized to large cells with polymorphous nuclei, prominent nucleoli and basophilic cytoplasm (Lymph node aspirate smears, May-Grünwald Giemsa ×1000)

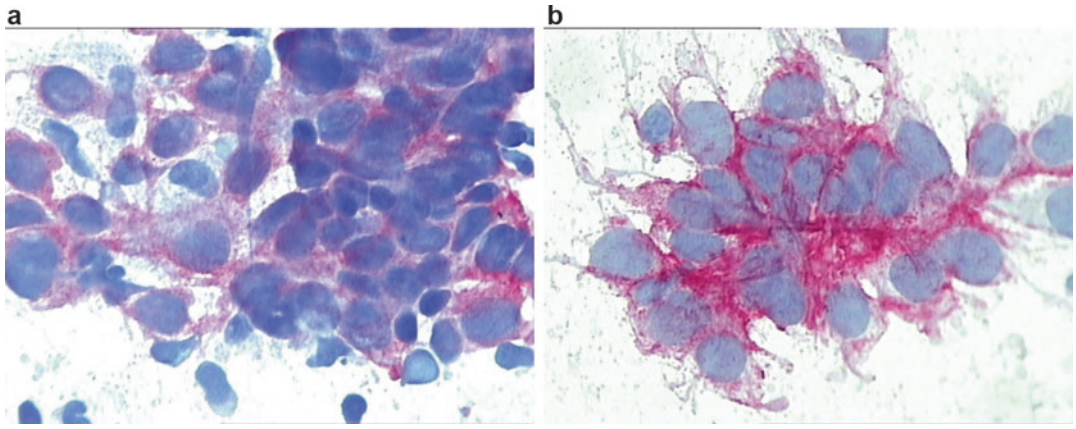


Viral Infections, Cytological Findings, Fig. 35 Non-Hodgkin lymphoma – Peripheral T-cell lymphoma. Small to medium sized, atypical lymphocytes with mostly irregular nuclei, eosinophils, and plasma cells infiltrating bone marrow (Bone marrow aspirate smear, May-Grünwald Giemsa ×1000)

infection (Fig. 29). EBV is also closely related with several forms of lymphomas as Hodgkin lymphoma (Figs. 30 and 31), Burkitt lymphoma (Figs. 32 and 33), T-cell lymphoma (Figs. 34 and 35), and nasopharyngeal carcinoma (Figs. 36 and 37). It is considered that virus gene products aimed at helping host cell to survive contribute to uncontrolled growth. Unfortunately, many other necessary steps in malignant transformation are not clear yet (Klein et al. 2007).



Viral Infections, Cytological Findings, Fig. 36 Lymph node metastasis of the nasopharyngeal carcinoma. (**a**, **b**) Large, atypical epithelial cells with irregular nuclei and multiple small nucleoli. Cytoplasms are fragile and poorly preserved. In the background are the lymphocytes (Lymph node aspirate smears, May-Grünwald Giemsa ×1000)



Viral Infections, Cytological Findings, Fig. 37 Lymph node metastasis of the nasopharyngeal carcinoma. Immunocytochemistry. (**a**) Cells immunostained for CD5/6 and (**b**) Pancytokeratin (Lymph node aspirate smears, LSAB ×1000)

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W

Warthin Tumor, Cytological Findings

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Synonyms

Cystadenolymphoma; Cystadenoma papilliferum; Papillary cystadenoma; Papillary cystadenoma lymphomatosum

Definition

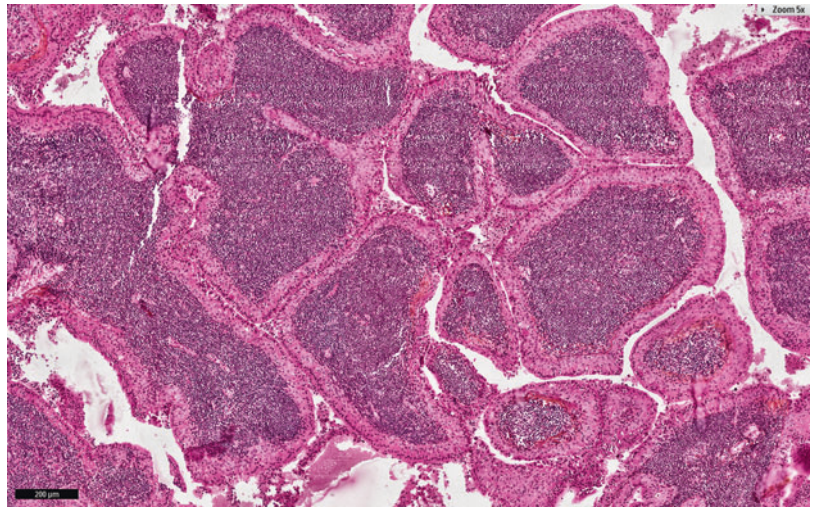
Warthin tumor (WT) is a benign neoplasm composed of bilayered oncocytic/columnar (external) and basal cell (internal) epithelium in papillary, cystic, and cribriform growth (Barnes et al. 2005; Ellis and Auclair 2008). The stroma is polymorphous lymphocytic, resembling stimulated lymph node (Fig. 1).

Oncocytoma (ON) is a benign tumor composed exclusively of oncocytic cells (Barnes et al. 2005; Ellis and Auclair 2008) without lymphocytic stroma (Fig. 2).

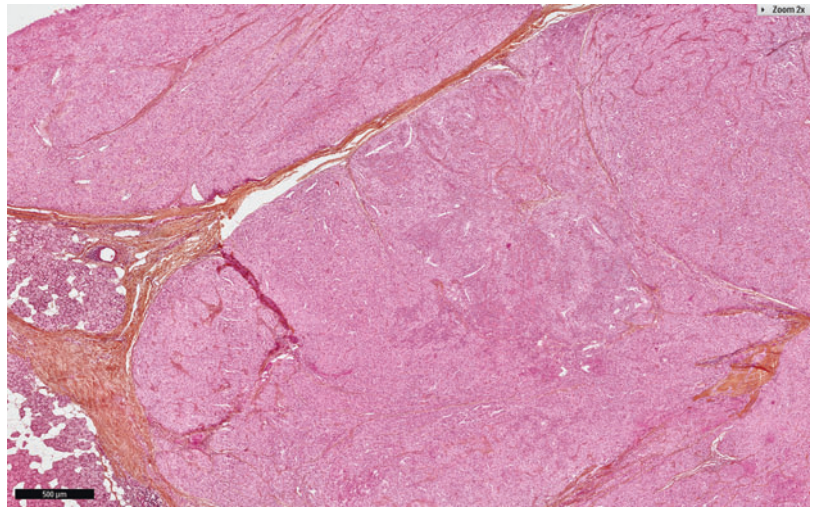
Clinical Features

- **Incidence**
  WT is the second most common salivary gland adenoma. It accounts for 5–25% of all salivary gland tumors. True ONs are exceptional and account for less than 1% of salivary gland tumors. Smokers have an elevated risk of WT as compared to nonsmokers' population.
- **Age**
  WT may occur at any age, including pediatric age. The pic incidence is between 50 and 80 years. ON has a pic of incidence between 60 and 90 years.
- **Sex**
  The classical ratio of male to female was 5 to 1, but was recently reduced to 1.6 to 1.
- **Site**
  Almost all WT and ON arise in parotid gland.
- **Treatment**
  Surgery is a treatment with a very low-rate of recurrences.
- **Outcome**
  Recurrences are exceptional.

F. Schmitt (ed.), *Cytopathology*, Encyclopedia of Pathology,
DOI 10.1007/978-3-319-33286-4



Warthin Tumor, Cytological Findings, Fig. 1 Warthin tumor. Bilayered oncocytic/columnar (external) and basal cell (internal) epithelium in papillary, cystic, and cribriform growth. The stroma is lymphocytic (H&E stain)



Warthin Tumor, Cytological Findings, Fig. 2 Oncocytoma. Oncocytic cells without lymphocytic stroma (H&E stain)

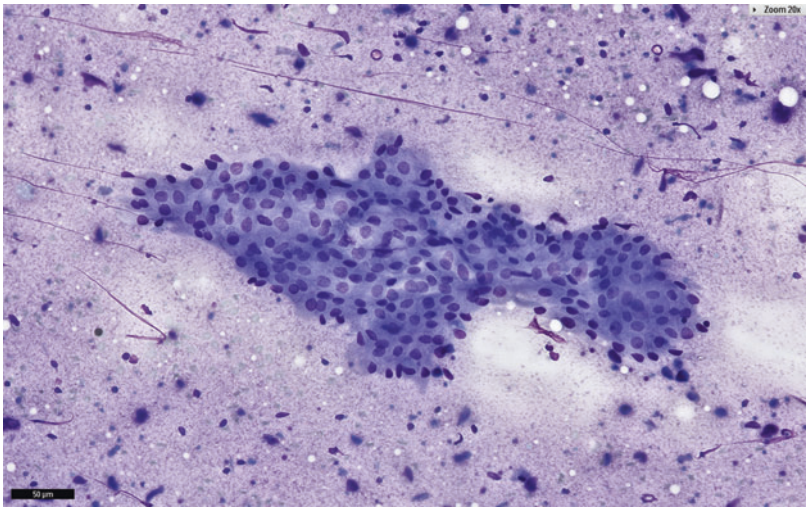
Macroscopy

WT is a well-encapsulated, polycystic grayish mass. Cystic component consists of brown, "dirty," and dense fluid. WT may be multifocal and/or bilateral.

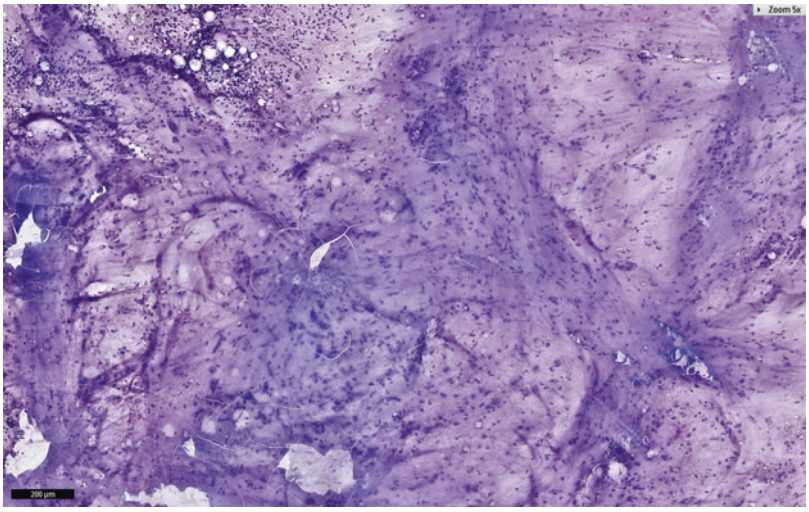
Microscopy

Smears in WT are usually hypercellular and cell rich and stroma rich (Klijanienko and Vielh 1997a). Smears in oncocytoma (ON) are also usually hypercellular but cell rich and stroma poor. WT and ON belong to the group of tumors exhibiting predominant oncocytic cell morphology.

Aspirates from WT usually consist of liquid. Three distinctive cytological components, either single or associated, are commonly present in different proportions: oncocytic cells, lymphocytic stroma, and necrosis or pseudo-necrosis (Fig. 3). Oncocytic cells are isolated or more frequently clustered or in papillary configurations with a polyhedral appearance and a large finely granular



Warthin Tumor, Cytological Findings, Fig. 3 Warthin tumor. Oncocytic cells, lymphocytic stroma, and pseudo-necrosis (MGG stain)



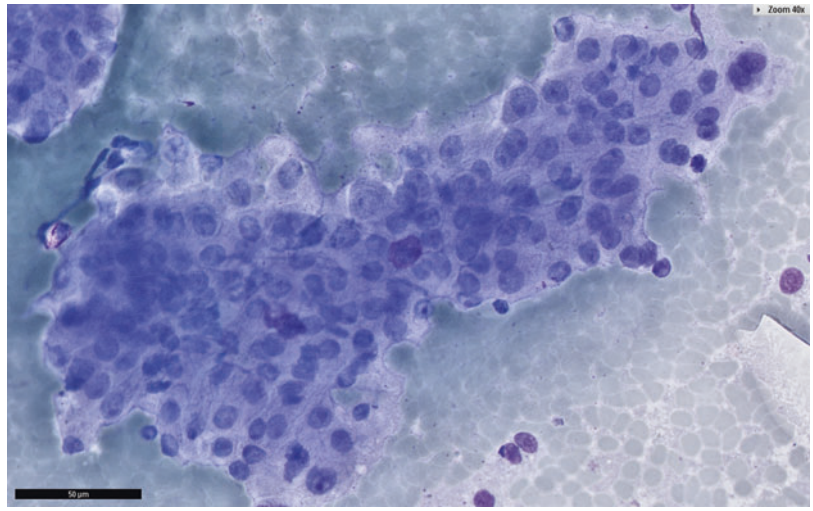
Warthin Tumor, Cytological Findings, Fig. 4 Warthin tumor. Necrosis resembling mucinous content in mucoepidermoid carcinoma (MGG stain)

cytoplasm. Mast cells are frequently seen within the clusters. Lymphocytic stroma is polymorphous mimicking a stimulated lymph node. Necrosis or pseudo-necrosis resembles necrosis in squamous cell carcinoma or mucinous content (macrophages, crystalloids) in mucoepidermoid carcinoma (Fig. 4). Occasionally, squamous cells, keratins debris, and sebaceous cells may be also observed. The accurate diagnosis is easy when all three components (clustered oncocytic cells with mast cells, lymphocytic background, and necrotic-like material) are present. However, smears are frequently less representative and may be composed of only one or two components (Klijanienko and Vielh 1997a).

Smears from ON show numerous oncocytes, usually in large clusters. Necrosis or lymphocytes are not detected (Fig. 5) (Verma and Kapila 2003).

Immunophenotype

There is no major interest to analyze WT or ON by immunohistochemistry. Oncocytic epithelium contains cytokeratin, CEA, and/or lactoferrin.



Warthin Tumor, Cytological Findings, Fig. 5 Oncocytoma. Oncocytes in large clusters. Necrosis or lymphocytes are not detected. Compare with Fig. 3 (MGG stain)

Molecular and Cytogenetic Features

Cytogenetic abnormalities include numerical and structural changes in 6p, 11q, and 19p regions.

Differential Diagnosis

When smears show only single component, the correct diagnosis of WT is difficult and the differential diagnosis should include intraparotid lymphadenitis or salivary cyst. When smears show exclusively oncocytes, WT should be differentiated with ON and acinic cell carcinoma (AcCC). Smears showing squamous cells or pseudo-mucoid necrosis should be differentiated from squamous cell carcinoma (SCC) and mucoepidermoid carcinoma (MEC).

Moderately or poorly differentiated AcCC may be composed of cells showing eosinophilic cytoplasm with microvacuolizations and lymphocytic background. This pattern may mimic oncocytic cells, and lymphocytes may mimic lymphocytic background in WT. In WT oncocytic clusters frequently contain mast cells which are absent in AcCC. The presence of well-differentiated acinic cells is against the diagnosis of WT (Klijanienko and Vielh 1997b).

The differential diagnosis between WT and SCC, and MEC may be difficult, especially when squamous metaplastic cells are numerous and when the background is necrotic. Oncocytes with mast cells are absent in SCC and MEC. On the other hand, some MECs may show eosinophilic roundish cells resembling oncocytes, but mucoid background and malignant squamoid/squamous cells are also present, confirming MEC (Klijanienko and Vielh 1997c; Klijanienko and Vielh 1998). Clinical evidence of cutaneous scalp or face squamous cell carcinoma will strongly argue in favor of the diagnosis of metastatic intraparotid tumor. Cytology is crucial, resolving this differential diagnosis problem, since, usually, WT exhibits a false-positive FDG PET/CT uptake (Klijanienko et al. 2012).

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This encyclopedia includes no entries for X, Y and Z.