Mieczyslaw Pokorski *Editor*

Respiratory Infections

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Editor
Mieczyslaw Pokorski
Institute of Psychology
University of Opole
Poland

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Preface

This is a new book series entitled Neuroscience and Respiration, a subseries of Springer's renowned Advances in Experimental Medicine and Biology. The book volumes present contributions by expert researchers and clinicians in the field of pulmonary disorders. The chapters provide timely overviews of contentious issues or recent advances in the diagnosis, classification, and treatment of the entire range of pulmonary disorders, both acute and chronic. The texts are thought as a merger of basic and clinical research dealing with respiratory medicine, neural and chemical regulation of respiration, and the interactive relationship between respiration and other neurobiological systems such as cardiovascular function or the mind-to-body connection. In detail, topics include lung function, hypoxic lung pathologies, epidemiology of respiratory ailments, sleep-disordered breathing, imaging, and biomarkers. Other needful areas of interest are acute respiratory infections or chronic inflammatory conditions of the respiratory tract, exemplified by asthma and chronic obstructive pulmonary disease (COPD), or those underlain by still unknown factors, such as sarcoidosis, respiratory allergies, lung cancer, and autoimmune disorders involving the respiratory system.

The prominent experts will focus their presentations on the leading-edge therapeutic concepts, methodologies, and innovative treatments. Pharmaco-therapy is always in the focus of respiratory research. The action and pharmacology of existing drugs and the development and evaluation of new agents are the heady area of research. Practical, data-driven options to manage patients will be considered. The chapters will present new research regarding older drugs, performed from a modern perspective or from a different pharmacotherapeutic angle. The introduction of new drugs and treatment approaches in both adults and children will be discussed. The problem of drug resistance, its spread, and deleterious consequences will be dealt with as well.

Lung ventilation is ultimately driven by the brain. However, neuropsy-chological aspects of respiratory disorders are still mostly a matter of conjecture. After decades of misunderstanding and neglect, emotions have been rediscovered as a powerful modifier or even the probable cause of various somatic disorders. Today, the link between stress and respiratory health is undeniable. Scientists accept a powerful psychological connection that can directly affect our quality of life and health span. Psychological approaches,

by decreasing stress, can play a major role in the development and course of respiratory disease, and the mind-body techniques can aid in their treatment.

Neuromolecular aspects relating to gene polymorphism and epigenesis, involving both heritable changes in the nucleotide sequence and functionally relevant changes to the genome that do not involve a change in the nucleotide sequence, leading to respiratory disorders will also be tackled. Clinical advances stemming from basic molecular and biochemical research are but possible if the research findings are "translated" into diagnostic tools, therapeutic procedures, and education, effectively reaching physicians and patients. All that cannot be achieved without a multidisciplinary, collaborative, "bench-to-bedside" approach involving both researchers and clinicians, which is the essence of the book series Neuroscience and Respiration.

The societal and economic burden of respiratory ailments has been on the rise worldwide leading to disabilities and shortening of life span. COPD alone causes more than three million deaths globally each year. Concerted efforts are required to improve this situation, and part of those efforts are gaining insights into the underlying mechanisms of disease and staying abreast with the latest developments in diagnosis and treatment regimens. It is hoped that the books published in this series will fulfill such a role by assuming a leading role in the field of respiratory medicine and research and will become a source of reference and inspiration for future research ideas.

Titles appearing in Neuroscience and Respiration will be assembled in a novel way in that chapters will first be published online to enhance their speedy visibility. Once there are enough chapters to form a book, the chapters will be assembled into complete volumes. At the end, I would like to express my deep gratitude to Mr. Martijn Roelandse and Ms. Tanja Koppejan from Springer's Life Sciences Department for their genuine interest in making this scientific endeavor come through and in the expert management of the production of this novel book series.

Opole, Poland   Mieczyslaw Pokorski

Volume 4: Respiratory Infections

The successful prophylaxis and treatment of ubiquitous respiratory infections is essential for the enhancement of public health. The chapters provide new insights into the biology of causative pathogens, tackle the epidemiological aspects, and present an update on diagnostics, prevention, and therapy of infections. The emerging new pathogens and antibiotic resistance of the old ones are discussed. Novel markers of the severity of community-acquired pneumonia, which bears high morbidity and mortality, are also presented.

Contents

Soluble Urokinase Plasminogen Activator Receptor: An Indicator of Pneumonia Severity in Children

A. Wrotek, T. Jackowska, and K. Pawlik

Abstract

Enhanced level of soluble urokinase plasminogen activator receptor (suPAR) level has been associated with activation of the immune system. It may be a novel biomarker for pneumonia severity, yet data on this subject are limited. In the present study we seek to determine the suPAR level in hospitalized children with community-acquired pneumonia (CAP), its correlation with pneumonia severity, and to compare the suPAR level between pneumonia and healthy conditions. The study encompassed a total of 596 children: 447 with pneumonia and 119 healthy. suPAR was measured in 227 out of the 447 pneumonia patients and in all healthy subjects. We used clinical indicators (fever, time for defeverscence, heart and breath rate, saturation, and length of antibiotic treatment and of hospitalization) and laboratory indicators (CRP, procalcitonin, white blood cell count, and sodium) to assess the CAP severity. The finding were that the suPAR concentration in children with pneumonia was significantly higher (median 7.11 ng/mL) than in healthy individuals (4.68 ng/mL). We found a positive correlation between the suPAR and the following factors: fever, time for defeverscence, length of hospital stay, and elevated CRP and procalcitonin levels. There was a reverse correlation with sodium concentration and capillary blood saturation. Moreover, the suPAR level was significantly higher in children with a severe course of pneumonia compared with those having non-severe pneumonia (7.79 vs. 6.87 ng/mL; $p = 0.006$). In conclusion, suPAR elevation is observed in pneumonia and may reflect its severity.

A. Wrotek, T. Jackowska (✉), and K. Pawlik
Department of Pediatrics, Medical Center of Postgraduate Education, 99/103 Marymoncka St., Warsaw 01-813, Poland

Department of Pediatrics, Bielanski Hospital, Warsaw, Poland
e-mail: tjackowska@cmkp.edu.pl

1   Introduction

Community-acquired pneumonia (CAP) is one of the major health concerns in children, being responsible for about 2.6 million cases of pneumonia in children under 5 years of age each year (Madhi et al. 2013), ending up with a million deaths (Liu et al. 2012). The highest percentage of children hospitalized due to pneumonia (approximately 50–60 %) concerns this age group (Madhi et al. 2013).

A search for new diagnostic markers is crucial for improving the evaluation of pneumonia, especially as no widely-used scale exists to rate the CAP severity in children, and the up-to-date guidelines regarding the indications for hospitalization are based rather upon a consensus than on evidence-based medicine. An ideal biomarker in pneumonia should be easy to measure, characteristic for pneumonia and should have both prognostic and predictive values. The soluble urokinase plasminogen activator receptor (suPAR) may be one such biomarker in pneumonia. The suPAR progenitor is the uPAR (urokinase plasminogen activator), which is bound to the surface of various cells, including the immune system cells, and plays an important role in cellular adhesion and migration, cell-stroma interaction, the induction of chemotaxis and proteolysis (Sidenius et al. 2000a, b; Plesner et al. 1997). After proteolytical cleavage, suPAR is released from the uPAR and forms a protein present in plasma, cerebrospinal fluid, or urine (Garcia-Monco et al. 2002; De Witte et al. 1998). Numerous studies have shown elevated suPAR levels under various conditions, such as viral (Ostrowski et al. 2004), parasitic (Ostrowski et al. 2005a, b), and bacterial infections (Eugen-Olsen et al. 2002; Garcia-Monco et al. 2002), but also in autoimmune (Toldi et al. 2013) and neoplastic diseases (Sier et al. 1998). Hence, suPAR increase is thought to reflect inflammatory activation, regardless of its source. The data published to-date show a prognostic value of suPAR in various pathological conditions, such as bacteremia (Huttunen et al. 2011), HIV infection (Ostrowski et al. 2005a, b; Sidenius et al. 2000a, b), or cancer

(Stephens et al. 1999). We previously reported that the suPAR correlates with the C-reactive protein (CRP) and procalcitonin (PCT) levels in children with CAP (Wrotek et al. 2013). The aim of the present study was to investigate the suPAR level in children with CAP, its correlation with pneumonia severity, and differences between healthy and ill children.

2   Methods

The study protocol was approved by a local Ethics Committee. This study, designed as a prospective trial, took place at the Department of Pediatrics of the Medical Center of Postgraduate Education and at the Bielanski Hospital in Warsaw, Poland. The study encompassed a total of 596 children, hospitalized between January 2011 and March 2013 due to pneumonia. To discern the community-acquired from nosocomial pneumonia, only children who were diagnosed with pneumonia up to 48 h after hospital admission were enrolled. The inclusion criteria also included a radiological confirmation of CAP. Among the exclusion criteria were: a previously diagnosed proliferative disease (even if the treatment was found to be completely successful), diabetes mellitus and organ insufficiency (cardiac, renal, adrenal, hypophyseal, or thyroidal), medical interventions, as well as musculoskeletal defects that might possibly affect the CAP course or contribute to a disease facilitating infection (such as tetraplegia, tracheostomy, or lung decortication), and lack of full information on the CAP course (e.g., patients discharged on parents' request). The blood samples were collected at admission and stored at $-70\,^{\circ}$C until the ELISA test (suPARnostic; ViroGates, Birkerød Denmark) was performed.

To create a control group, healthy children at a corresponding age hospitalized at the Department of Pediatrics due to spurious or non-infectious causes (like suspicion of intoxication which was later excluded, stomachache, or chest pain with a dominant psychological component) were recruited. To exclude an asymptomatic or chronic inflammatory disease, the

high-sensitive C-reactive protein (hsCRP) concentration was measured in each patient, and only children with hsCRP levels within the normal range were enrolled. Likewise, the exclusion criteria included a previously diagnosed proliferative disease, diabetes mellitus, organ insufficiency, or any other known pathological condition.

The patient enrollment scheme is shown in Fig. 1. There were a total of 477 pneumonia cases; 65 of them failed to meet the inclusion criteria and thus were excluded from further analysis. Out of the 412 pneumonia patients included in the study, 227 (114 females, 113 males) aged 8 days to 18 years (median 38 months) were randomly chosen for the suPAR measurement. Based on the serum inflammatory markers CRP and PCT, the children were classified as severe pneumonia (elevation of both CRP and PCT) or non-severe pneumonia (elevation of either marker). Additionally, since CAP may be accompanied by hyponatremia (HN), which may affect both suPAR concentration and the course of pneumonia, the children were stratified into those with lowered and normal sodium levels. A hundred and nineteen (60 females, 59 males) healthy children aged 7 days to 18 years (median age 49 months) formed the control group (group C). There were no differences among the groups concerning the children's gender or age (p = 0.38).

Clinical features (body temperature on admission, time for defeverscence, heart rate, breath rate, capillary blood saturation, length of antibiotic course and hospital stay), as well as serum inflammatory markers (CRP, PCT, white blood cell count, and neutrophil percentage) were used to assess the CAP severity. Because of physiological age-dependent differences in cardio-respiratory functions, breath frequency and heart rate were analyzed separately in children under and over 1 year of age. Likewise, white blood cells count and neutrophil count were analyzed separately in children under and over 4 years of age.

To describe continuous variables, means ±SD, for normally distributed data, and medians with interquartile range (IQR), for skewed data,

were used. The Shapiro-Wilk test was used to determine the data distribution. A t-test or Mann-Whitney U test was performed for data comparisons as required. Correlations between suPAR and clinical markers were assesses with Spearmann's rank correlation coefficient. $p < 0.05$ was defined as the level of statistical significance. A commercial STATISTICA ver. 10 (StatSoft) package was used for data elaboration.

3　　Results

The median suPAR concentration in children with CAP was 7.11 ng/mL (IQR: 5.47–8.95), while in healthy individuals it was 4.68 ng/mL (IQR: 3.68–5.91); the difference was statistically significant ($p < 0.001$) (Fig. 2a). When the groups were broken down by the severity of CAP and by the presence of hyponatremia, suPAR levels were significantly greater in the severe and hyponatremic subgroups. The median suPAR in severe vs. non-severe CAP were 7.79 ng/mL (IQR: 6.55–9.69) vs. 6.87 ng/mL (IQR: 5.28–8.53) ($p < 0.01$) (Fig. 2b) and in CAP complicated by hyponatremia it was 7.57 ng/mL (IQR: 6.20–9.64) vs. 6.92 ng/mL (IQR: 5.10–8.42) (p = 0.01) in non-hyponatremic children (Fig. 2c).

There was a positive correlation between suPAR and clinical as well as laboratory markers of disease severity, such as fever, time for defeverscence, and length of hospital stay ($r = 0.21$, $r = 0.31$, and $r = 0.21$, respectively). A negative correlation was between suPAR and capillary blood saturation ($r = -0.16$), which also reflects the pneumonia severity. The correlations remained irrelevant for breath rate, heart rate, or length of antibiotic treatment (Table 1). However, the level of suPAR correlated positively with CRP and PCT ($r = 0.15$ and $r = 0.29$, respectively) and with the white blood cell count in children under 4 ($r = 0.25$), and negatively with the sodium concentration ($r = -0.23$). There was no association between suPAR and neutrophil or lymphocyte percentage, nor was there any between suPAR and the white blood cell count in children over 4 years old.
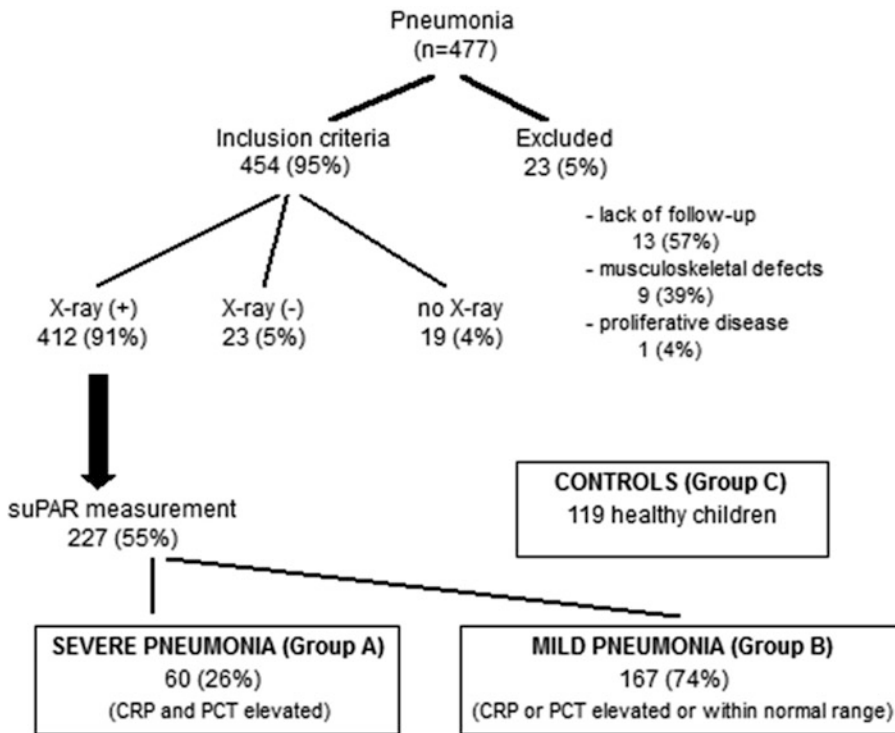
Fig. 1 Patients enrollment scheme
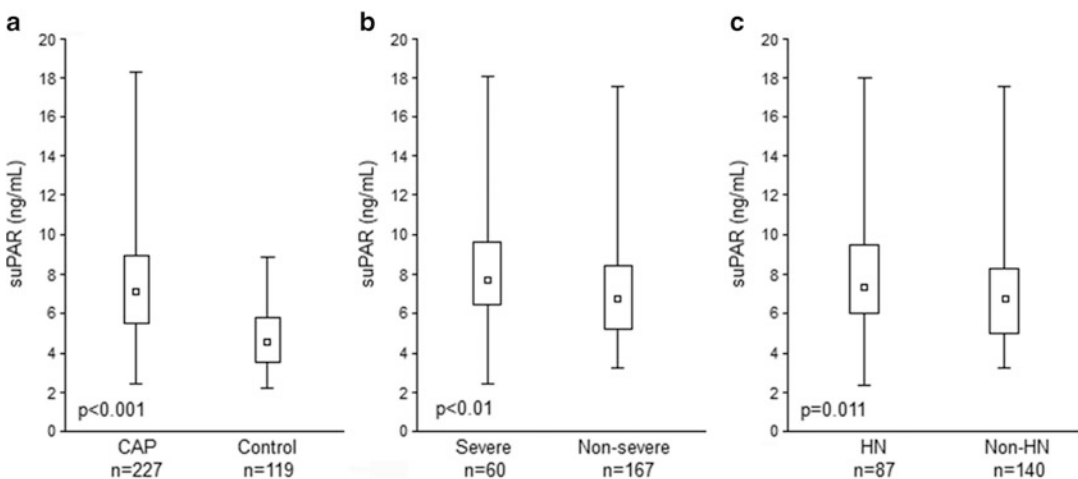
Fig. 2 suPAR in children with community acquired pneumonia (CAP) compared with healthy individuals (**a**), in CAP broken down by the severity of disease (**b**) and by the level of natremia (**c**). *n* number of patients, *box plots* with lower quartile (LQ) and upper quartile (UQ) bars, *HN* hyponatremic, *non-HN* non-hyponatremic patients; differences assessed with Mann-Whitney U test

Table 1 Characteristics of children with pneumonia and Spearmann's correlation rank coefficient (r) between the suPAR and selected parameters

Characteristics	Median	LQ–UQ	r
Length of hospitalization (days)	7.0	5.0–9.0	0.21
Fever (°C)	38.4	37.3–39.0	0.21
Defeverscence (h)	36.0	12.0–60.0	0.31
Breath rate (per min)	30.0	20.0–44.0	NS
Capillary blood saturation (%)	96.0	94.0–97.0	−0.16
Heart rate (per min)	120.0	100.0–135.0	NS
Antibiotic treatment (days)	10.0	8.0–10.0	NS
CRP (mg/dL)	22.9	7.0–66.8	0.15
PCT (ng/mL)	0.2	0.1–0.9	0.29
Sodium (mmol/L)	136.0	135.0–138.0	−0.23
WBC ($\times 10^3/\mu$L)	12.5	8.5–17.5	NS
Neutr. (%)	61.0	48.0–71.0	NS
Lymph. (%)	27.0	19.0–39.0	NS
Under 4 years old			
WBC ($\times 10^3/\mu$L)	13.3	9.6–17.8	0.25
Neutr. (%)	56.0	41.0–67.0	NS
Lymph. (%)	32.0	22.0–48.0	NS
Over 4 years old			
WBC ($\times 10^3/\mu$L)	10.4	7.4–15.7	NS
Neutr. (%)	69.0	61.0–79.0	NS
Lymph. (%)	22.0	12.0–28.0	NS

Spearmann's correlation rank coefficient – values shown only for statistically significant coefficients
LQ lower quartile, *UQ* upper quartile, *WBC* white blood cells count, *Neutr.* neutrophil percentage, *Lymph.* lymphocyte percentage

4 Discussion

The current study showed that suPAR levels are increased in children with pneumonia and are related with CAP severity. The median suPAR concentrations in children with CAP were much higher than those observed in healthy individuals, although the suPAR levels in the control group of this study were somewhat higher than in the controls of other studies. Wittenhagen et al. (2011) reported the median plasma levels in healthy children to be 2.3 ng/mL. These differences may primarily result from the control group selection. Importantly, suPAR values in children with and without CAP overlapped only in a small number of patients ($p < 10^{-6}$, Mann Whitney U test). This makes the suPAR a possible novel biomarker of pneumonia in children, a marker that might be helpful in differentiating children with CAP from healthy individuals. To our knowledge, except for our previously published pilot study (Wrotek et al. 2013), this is the first study on suPAR levels in children with CAP. Future studies comparing children with radiologically confirmed pneumonia versus upper-respiratory tract infection or bronchitis may have an influence on the practical use of suPAR, for example, at the emergency room. For the time being, the issue of the suPAR diagnostic usefulness remains unresolved as it has been reported to add little value to the diagnostic process in adult septic patients (Backes et al. 2012).

There is not enough data to fully explain the role of suPAR and its possible association with pulmonary processes. Yet, studies that have been performed in a mouse model may clarify the potential pathway. The uPAR, a precursor of suPAR, is required to recruit neutrophils toward

the lungs in *Pseudomonas aeruginosa* pneumonia (Gyetko et al. 2000). Phagocytosis and generation of superoxide are diminished in uPAR-knockout mice (Gyetko et al. 2004). Also, uPAR-knockout mice demonstrate a decreased neutrophil migration in the lungs (Wiersinga et al. 2010). Therefore, individuals with an impaired uPAR (or suPAR) system may show decreased effectiveness in the neutrophil-mediated bactericide effect. However, the previously hypothesized occurrence of uPAR deficiency in humans has not yet been reported (Wrotek et al. 2013). Likewise, uPAR affinity to the pulmonary compartment remains hypothetical.

The principal finding of the present study may be a correlation between suPAR and pneumonia severity. None of the Spearmann's rank coefficients presented above should be considered as clinically important due to their relatively low power. However, it should be emphasized that suPAR correlates with almost every of the analyzed and widely-used pneumonia severity parameters, which makes it useful as a prognostic tool. Several studies have indicated an unfavorable outcome in patients with elevated suPAR levels, in case of malaria (Ostrowski et al. 2005a, b) or tuberculosis (Eugen-Olsen et al. 2002). The prognostic value of suPAR as a single marker in critically ill patients has been found to be higher than those of other routinely used biomarkers, such as CRP or procalcitonin (Kofoed et al. 2008). This value of suPAR increases when combined with other biological and clinical markers, forming a part of more sophisticated systems that are currently used to stratify the severity of disease (Backes et al. 2012). The suPAR seems to carry a potential to become a key component of future assessment scales of severity of community-acquired pneumonia.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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Detection of Respiratory Tract Pathogens with Molecular Biology Methods

A. Wozniak-Kosek, J. Kosek, and B. Zielnik-Jurkiewicz

Abstract

This paper describes the use in routine diagnosis of virological kit, which was designed to identify the 15 most common respiratory viruses in clinical specimens of nasopharyngeal aspirates, swabs, and bronchoalveolar lavage. It is a one-step multiplex RT-PCR system for the detection of influenza virus type A and type B, human respiratory syncytial virus type A, B; human adenovirus, human metapneumovirus, human coronaviruses 229E/NL63 and OC43, human parainfluenza type 1, 2, 3, human rhinovirus type A, B, human enterovirus, and bocavirus 1, 2, 3, 4. The article presents research conducted on the basis of swabs collected from patients who came to the Ear, Nose, and Throat Emergency Care Unit at the Department of Otolaryngology, Military Medical Institute in Warsaw, in February 2013. Due to the nature of work in an laryngological emergency ward, the material was collected only from those patients who reported problems associated with rhinitis or any dysfunction of the upper respiratory tract. The study shows that patients who came to seek laryngological assistance were usually infected with viruses having affinity for the airway epithelium.

Keywords

Diagnostics • Infection • Polymerase chain reaction • Respiratory virus

A. Wozniak-Kosek (✉)
Department of Influenza Research, National Influenza Center, National Institute of Public Health-National Institute of Hygiene, 24 Chocimska St., 00-791 Warsaw, Poland
e-mail: kaj12@poczta.fm

J. Kosek
Otolaryngology Clinic, Military Institute of Medicine, Warsaw, Poland

B. Zielnik-Jurkiewicz
Ear, Nose, and Throat Department, Children's Hospital, Warsaw, Poland

1 Introduction

The laboratory diagnostics of virological infections in the respiratory system is currently based primarily on modern methods of molecular biology. Serological methods, and in the case of influenza virus infection also a viral culture in chick embryo, constitute a smaller value for diagnostics, because of the time needed to obtain a result, although they are still considered to be the gold standard in virological studies of

the respiratory system. The respiratory tract infection is divided into upper and lower respiratory tract infection. However, the upper respiratory tract infections constitute a preliminary stage to the infection of the bronchi and lung parenchyma. Chronic heart and lung diseases, diabetes, cancer, renal failure, hematopoietic system diseases, and other chronic diseases occur more frequently as complications in the case of patients with severe lower respiratory tract infections involving the respiratory viruses (Kim et al. 2013). In such a case, especially when the patient with severe symptoms of respiratory tract infection and associated immune disorders or the comorbidities comes to the laryngological ER, the virological laboratory diagnostics allowing for the identification of the etiological agent is extremely helpful.

1.1   Selected Pathogens Responsible for Respiratory Tract Infections

Respiratory tract infections are the most frequent cause of visits to the doctor. Pneumonia is still a threat to life and health, especially in children and elderly patients affected by comorbidities. The pathogens responsible for respiratory infections include viruses, bacteria, and in exceptional cases, fungi and parasites. Table 1 shows the most common viruses that are the cause of respiratory tract infections.

2   Methods

The material consisted of swabs from the nose and throat collected from patients during the ear, nose, and throat (ENT) emergency care in the period January–February 2013 in the Military Medical Institute in Warsaw, Poland (14 samples), and of the specimens which were accepted for tests for the detection of respiratory viruses by the Laboratory of Influenza Virus Research, National Center for Influenza in the National Institute of Public Health-National Institute of Hygiene in the season 2012/2013 (10 samples). The material was collected no later than 5 days after the onset of clinical symptoms according to the accepted criteria (WHO 2011).

2.1   Isolation of Viral RNA

The isolation of RNA is a template used in further polymerase chain reaction (PCR) studies to detect the presence of genetic material of viruses that cause respiratory tract infections. For this purpose QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used. The kit is designed to isolate RNA from clinical samples. A single elution with the use of elution buffer is sufficient to recover at least 90 % of the viral RNA from the QIAamp column.

2.2   Carrying Out a PCR Reaction

The RV15 One Step ACE Detection Kit (Seegene, Seoul, South Korea) is a qualitative *in vitro* test for the detection of 15 types of respiratory viruses in the aspirates from nasopharynx and nasopharyngeal swabs or samples from bronchopulmonary tree lavage in patients with clinical symptoms. The kit contains a set of reagents due to which it is possible to perform a Multiplex PCR. Simplification of the two-step method results in an increased repeatability of analysis and efficiency of reaction. The kit for determining 15 respiratory viruses in a sample of a clinical material is based on a process of reverse transcription (RT) and amplification of the target DNA by PCR using Dual Priming Oligonucleotide (DPO) primers, which provides freedom in a primer design and PCR optimisation and maximizes PCR specificity and sensitivity by fundamentally blocking non-specific priming. The DPO-based multiplex assay that permits the simultaneous amplification of target sequences of 15 viruses, presented after the amplification reaction, uses e.g., electrophoresis in an agarose gel preceded by nucleic acid isolation (Kim et al. 2013).

The kit consists of three panels/sets A, B, C, each containing reagents and primers for the detection of five respiratory viruses (details are given in Table 2) and includes two internal controls: PCR control and the control of the whole process. PCR controls were added to the A and B panel in order to identify the substances

Table 1 Basic clinical and diagnostic methods in selected infections caused by respiratory viruses (Brydak 2008)

Virus type	Family	Basic clinical symptoms	Diagnostic methods
Influenza virus type A and B	*Orthomyxoviridae*	Fever, chills, muscle pain and headache, rhinitis, conjunctivitis, and inflammation of the tonsils, throat, and larynx.	Molecular methods, virus isolation in chick embryo or MDCK cell culture, antigen detection using IF immunofluorescence test, ELISA. Serological methods such as hemagglutination inhibition reaction OZHA.
Human parainfluenza virus type 1–4	*Paramyxoviridae*	Fever, cough, hoarseness, upper respiratory tract infection, bronchitis, and pneumonia.	Molecular methods, isolation in a continuous human cell line, such as HeLa-CPE; after 2–10 days antigen detection with F test, OZHA serological methods, and ELISA.
Human respiratory syncytial virus type A and B	*Paramyxoviridae*	Fever, upper respiratory tract inflammation, bronchitis, and pneumonia.	Molecular methods, isolation in a continuous human cell line, such as HeLa-CPE; after 2–10 days antigen detection with F test, OZHA serological methods, and ELISA.
Human rhinovirus type A–C	*Picornaviridae*	Sneezing, runny nose, cough, sore throat, headache, and less frequently fever.	Benign course and thus, frequently only clinical diagnosis or research using molecular biology techniques.
Human adenovirus	*Adenoviridae*	Chills, fatigue, high temperature, runny nose, dry cough, and inflammation of the glands in the neck.	Molecular methods. Isolation of the virus in the cell line HeLa or Hep-2, antigen detection with IF test.
Human coronavirus	*Coronaviridae*	Runny nose, sneezing, sore throat, high temperature, chills, headache, and inflammation of the lymph nodes.	Poorly replicating virus in cell culture, requiring human embryonic tracheal organ cultures or nasal epithelium; therefore molecular methods are recommended.
Human enterovirus	*Picornaviridae*	Pharyngitis, pneumonia.	Molecular methods, virus isolation in cell culture, serological methods, such as neutralization reaction.
Human metapneumovirus	*Paramyxoviridae*	Exacerbations of chronic inflammatory diseases of the respiratory system in children and adults.	Molecular methods.
Human bocavirus	*Parvoviridae*	Exacerbation of respiratory system diseases especially in infants and young children.	Molecular methods.

contained in the tested samples and to determine whether they might interfere with PCR amplification. Panel C has as an internal control a human RNase P, which allows the inspection of the whole process from the extraction of nucleic acids to RT-PCR. Additionally, 8-methoxypsoralen was used, which suppresses the activity of a DNA template. The 8-methoxypsoralen (8-MOP) reagent binds to the double-stranded structure of nucleic acids, forming covalent bonds between the strands upon activation by ultraviolet light.

2.3 Detection of PCR Products by Electrophoresis in Agarose Gel

When one-stage PCR was carried out in a thermocycler, the amplification products were placed onto a previously prepared 1.5 % agarose gel. The preparation of the gel consisted of dissolving the agarose in TAE (Tris-acetate-EDTA) buffer. After partial cooling, ethidium bromide was added at a concentration of 10 mg/ml. The gel was solidified using special well combs and mixtures, after amplification, were

Table 2 Amplicon information

RV 15 One Step ACE Detection (A set)		Size in agarose gel (bp)[a]
PCR control		850
Human adenovirus	ADV	534
Human coronavirus 229E/NL63		375
Human parainfluenza virus 2	PIV1	264
Human parainfluenza virus 3	PIV2	189
Human parainfluenza virus 1	PIV3	153
RV 15 One Step ACE Detection (B set)		Size in agarose gel (bp)
PCR control		850
Human coronavirus OC43		578
Human rhinovirus A/B/C	HRV	394
Human respiratory syncytial virus A	RSV A	269
Influenza A virus		206
Human respiratory syncytial virus B	RSV B	155
RV 15 One Step ACE Detection (C set)		Size in agarose gel (bp)
Human bocavirus 1/2/3/4	HBoV	579
Influenza B virus		456
Human metapneumovirus	MPV	351
Human parainfluenza virus 4	PIV4	254
Human enterovirus	HEV	194
Whole process		153

[a]Size of the linear DNA that can be resolved in agarose gel (bp)

applied to the resulting holes in the agarose. The mixture also contained a loading dye. This procedure makes it possible to control the electrophoretic separation process using the kit for electrophoresis Wide Mini-SubCell GT/PowerPac Basic System (Bio-Rad Laboratories, Hercules, CA). Thus, the electrophoresis was carried out by controlling the migration of PCR products in a gel by placing a loading dye – bromphenol blue. To get the maximum separation of DNA fragments, the electric field did not exceed 5 V/cm of the gel. After the electrophoresis, the gel was analyzed under UV light in the GelDoc EQ system, and the resulting image was documented using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

2.4 Eligibility Criteria of the Test

The presence of 15 respiratory viruses in the RNA samples tested was confirmed by the amplification products of the proper base pairs, as shown in Table 2.

3 Results and Discussion

We found genetic material of eight different respiratory viruses including: Influenza Virus type A and B, Human enterovirus, Human parainfluenza virus, Human rhinovirus, Human coronavirus OC43, Human respiratory syncytial virus, and Human metapneumovirus. A co-infection of two or three types of respiratory viruses was often present. Of the 24 samples taken from patients, there were mixed infections with respiratory viruses in 15 cases (62.5 %); the most common co-infection combinations are presented in Table 3. Of the remaining nine samples, only viral genetic materials were identified of: influenza virus type A in 7 (29.1 %) cases, and Coronavirus OC43 in one and HEV virus in one case each. Figure 1 shows a breakdown of viruses.

Virological laboratory methods can be a valuable addition to medical clinical diagnostics conducted in patients with respiratory tract infections and avoid the use of unnecessary antibiotic therapy (Brydak et al. 2013). The commissioning of a microbiological examination must be supported by an appropriate medical decision on whether and how the result will be used. The selection of, or resignation from, the available laboratory testing method should be based on the analysis of the workload, cost, waiting time for the outcome, and the expected clinical benefits for patients (Wozniak-Kosek and Bydak 2013).

The knowledge of available laboratory techniques both virological and bacteriological, and their advantages and limitations is important for decision-making (Nitsch-Osuch et al. 2013). In cases requiring a rapid response on the part of

Table 3 Genetic material of respiratory viruses in nasal and throat swabs taken from hospital patients from Mazovian Voivodeship in Poland

Genetic material of viruses
Influenza virus type A; RSV A; PIV 3, PIV 2
Influenza virus type A; HRV
Influenza virus type A; RSV A, HEV
Influenza virus type A; RSV A
Influenza virus type A; PIV-3, HEV
Influenza virus type A; HEV
Influenza virus type B; MPV
Coronavirus OC 43; RSV A
PIV1; RSV B; HEV
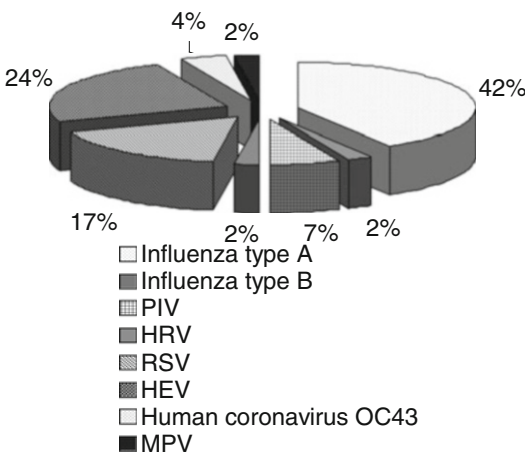
Fig. 1 Breakdown of respiratory viruses identified in throat and nose swabs

the laboratory staff, the collaboration with a doctor is crucial to select appropriate tests and to interpret results. The difficulty in the treatment of infections caused by respiratory viruses is related to the lack of effective drugs. The only exception to this end is the treatment of influenza with the neuraminidase inhibitors oseltamivir and zanamivir which are highly effective (Demkow 2008; Van-Tam and Sellwood 2013).

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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Classical Against Molecular-Genetic Methods for Susceptibility Testing of Antituberculotics

I. Porvaznik, J. Mokry, and I. Solovic

Abstract

Tuberculosis currently belongs to rare respiratory diseases in Slovakia. However, the emergence and spread of multi-drug resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are major challenges for global tuberculosis control, since the treatment of resistant forms creates both medical and financial problems. Cultivation methods of diagnosis are time-consuming, many times exceeding the time of the initial phase of tuberculosis treatment. Therefore, in the presented study we compared the standard procedures, based on the cultivation of mycobacteria and subsequent drug susceptibility testing to antituberculotics, with molecular-genetic methods using PCR diagnostic kits. The molecular-genetic testing enables to obtain direct and fast evidence of *Mycobacterium tuberculosis*, with genomic verification of resistance to the most important anti-tuberculosis drugs – isoniazid and rifampicin in MDR-TB, and ethambutol, aminoglycosides, and fluoroquinolones in XDR-TB. In 2012–2013, we confirmed 19 cases of drug-resistant tuberculosis in Slovakia. The resistance to rifampicin was confirmed in all strains with both methods. In two cases, the molecular-genetic testing did not show resistance to isoniazid, as confirmed by conventional cultivation. Furthermore, two strains demonstrating susceptibility in conventional microbiological testing to ethambutol and five strains to fluoroquinolones were verified as actually being resistant using a PCR method. Rapid diagnosis and identification of MDR-TB or XDR-TB strains using molecular-genetic testing is an essential tool for the timely and appropriate drug treatment and prevention of spread of drug resistant strains.

I. Porvaznik (✉)
National Reference Laboratory for Mycobacteria, National Institute of Tuberculosis, Pulmonary Diseases and Thoracic Surgery, 1 Vysne Hagy St., 059 84 Vysoke Tatry, Slovakia

Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia
e-mail: porvaznik@hagy.sk

J. Mokry
Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

I. Solovic
National Reference Laboratory for Mycobacteria, National Institute of Tuberculosis, Pulmonary Diseases and Thoracic Surgery, 1 Vysne Hagy St., 059 84 Vysoke Tatry, Slovakia

1 Introduction

Tuberculosis (TB) belongs to the rarest respiratory diseases in Slovakia, a country with a low incidence of TB. In 2012, there were 345 cases of tuberculosis reported in the National Register of TB (NRT 2012), which is 6.4/100,000 population. Bacteriologically confirmed were 191 cases (55.4 %). The number of new cases was 292 and the recurrence of tuberculosis was in 53 cases. Pulmonary TB was present in 298 cases and extrapulmonary TB in 47 cases.

In the national reference laboratory we bacteriologically confirmed 169 cases of TB in 2012. The resistance to antituberculotic drugs was confirmed in 13 cases, it was mostly non-resistance to isoniazid. Multi-drug resistant tuberculosis (MDR-TB) was confirmed in 4 cases. The incidence of resistance of mycobacterial strains to various anti-tuberculotic drugs is on the rise worldwide (Skrahina et al. 2012). The former Soviet Union countries belong to the areas where the resistant strains appear often. As immigrants often use Slovakia as a transit country, the problem of resistant TB strains may not be underestimated (Fig. 1).

1.1 Drug Resistance to Antituberculotics

The resistance of *Mycobacterium tuberculosis* to anti-tuberculotic drugs is mostly based on spontaneous random genetic mutations, typically occurring at a rate ranging from 3×10^{-7} to 1×10^{-8} per organism per generation for the first-line antituberculotic drugs (isoniazid, rifampicin, ethambutol, and streptomycin). From the molecular perspective, the resistance is based on gene mutations in mycobacteria, which frequently leads to a change in the target molecule making it functionally insensitive to the action of antituberculotic agents.

Telenti et al. (1993) described the molecular mechanism of rifampicin resistance in *M. tuberculosis*. Rifampicin acts by binding to a beta-subunit of the RNA polymerase (coded by the rpoB gene), inhibiting RNA transcription (Drobniewski and Wilson 1998). Subsequent DNA sequencing studies have shown that more than 95 % of rifampicin resistant strains have mutations in the 81-base pair region (codons 507–533) of the rpoB gene (Bartfai et al. 2001). More than 50 mutations within this region
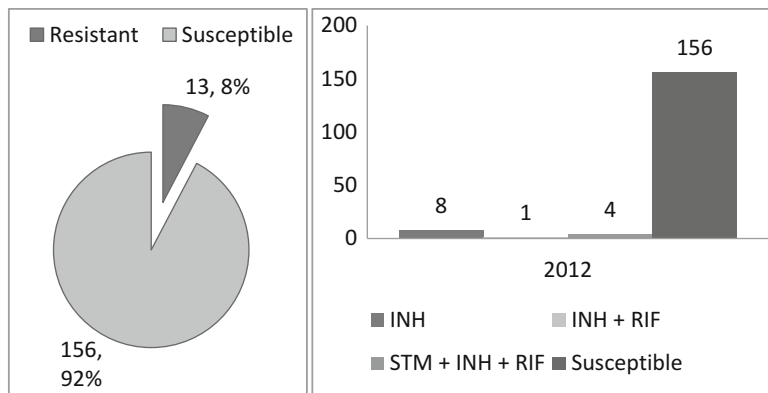

Fig. 1 Drug resistance in Slovakia in 2012. *INH* isoniazid, *RIF* rifampin, *STM* streptomycin

have been characterized by automated DNA sequencing; however, the majority are point mutations in codons 516, 526, or 531 (Gillespie 2002). Mutations in other regions of the rpoB gene have also been reported, but much less commonly. In addition, a few silent mutations infrequently occur which do not seem to confer rifampicin resistance.

Isoniazid inhibits InhA, enoyl-ACP-reductase, which is involved in the biosynthesis of mycolic acid (Gillespie 2002). Mutations causing isoniazid resistance are located in different regions of several genes. Isoniazid is a so-called 'pro-drug' which is converted to its active form by the catalase-peroxidase enzyme KatG. Therefore, resistance can be due to several factors, including the binding of the prodrug to its InhA target, the activation of the prodrug by KatG (encoded by the katG gene), or by increased expression of the target InhA (Miotto et al. 2008). Mutations in codon 315 of the katG gene have been found in 50–90 % of isoniazid resistant strains, while 20–35 % of isoniazid-resistant strains have been reported to have mutations in the inhA regulatory region, and 10–15 % of strains have mutations reported in the ahpC-oxyR intergenic region (Miotto et al. 2008; Brossier et al. 2006). Deficiency in catalase activity leads to high-level resistance to isoniazid. Most commonly, mutations in the katG gene are single point mutations at codon 315 involving a serine-to-threonine amino acid substitution. Other mutations in katG occur less commonly. Low-level resistance to isoniazid is mostly caused by inhA and ahpC-oxyR mutations (Miotto et al. 2008).

Ethambutol (EMB) is a narrow-spectrum antimycobacterial agent that is used for the treatment of tuberculosis. EMB is a first-line anti-tuberculosis agent that is especially important when used in drug combinations to prevent the emergence of drug resistance or to treat single drug-resistant tuberculosis (WHO 1997). Furthermore, streptomycin has been replaced by EMB as a key drug in the intensive phase of tuberculosis chemotherapy, as it is less expensive and patient compliance is better with this drug (Rabarijaona et al. 1999). This agent has been proposed to be an arabinose analog; the specific target is likely to be an arabinosyltransferase, presumably a functionally important site. A two-gene locus (embAB) that encodes arabinosyltransferase has been studied to elucidate a potential mechanism of EMB resistance (Alcaide et al. 1997; Telenti et al. 1997).

Fluoroquinolones are bactericidal antibiotics currently in use as second-line drugs in the treatment of TB. In M. tuberculosis, only type II topoisomerase (DNA gyrase) is present and thus is the only target for fluoroquinolone activity (Aubry et al. 2004). Type II topoisomerase is a tetramer composed of two A and B subunits, encoded by the genes gyrA and gyrB, respectively, which catalyses the supercoiling of DNA (Drlica 1999). Initial studies performed in laboratory strains of M. tuberculosis and M. smegmatis showed that resistance to fluoroquinolones is a result of aminoacid substitutions in the putative fluoroquinolone binding region in gyrA or gyrB.

Kanamycin and amikacin are aminoglycoside antibiotics, while capreomycin and viomycin are cyclic peptide antibiotics. All four are used as second-line drugs in the treatment of MDR-TB. Although belonging to two different antibiotic families, all exert their activity at the level of protein translation. Cross-resistance among kanamycin, capreomycin, and viomycin has been reported since the studies performed by Tsukamura and Mizuno (1975). The most common molecular mechanism of drug resistance has been associated with the A1401G mutation in the rrs gene coding for 16S rRNA. This mutation occurs more frequently in strains with high-level resistance to kanamycin and amikacin (Jugheli et al. 2009).

Traditionally, patients with MDR-TB are classified into two major groups: (1) those who acquired drug-resistant strain from community and (2) those with drug resistance developed due to previous therapy of tuberculosis. Only the cases of primary drug resistance are assumed to be due to transmission of drug-resistant strains (Van Rie et al. 2000). A person with fully susceptible TB may develop secondary (acquired) resistance during therapy. However, the clinical

term 'acquired' drug resistance should be replaced with 'drug resistance in previously treated cases', which includes cases with drug resistance due to true acquisition as well as that due to transmitted drug-resistant strains (Van Rie et al. 2000).

1.2 Classification of Drug Resistance

Generally, the resistance to antituberculotic drugs can be classified as follows (van der Werf et al. 2012):

- Mono-resistance – resistance to one of first line drugs;
- Poly-resistance – resistance to two and more drugs;
- Multi-resistance – resistance at least to isoniazid and rifampicin;
- XDR – extensively drug-drug resistant – resistance at least to isoniazid and rifampicin and to any fluoroquinolone or to any of the three second-line injectables (amikacin, capreomycin, and kanamycin).

The terms 'extremely drug resistant' (XXDR-TB) and 'totally drug-resistant TB' (TDR-TB) are also used by some authors (Cegielski et al. 2012). Nevertheless, TDR-TB is yet to be clearly defined. While the concept of TDR-TB is easily understood in general terms. In practice, however, *in vitro* drug susceptibility testing (DST) is technically challenging and limitations on the use of results remain (Cegielski et al. 2012). Conventional DST for the drugs that define MDR-TB and XDR-TB has been thoroughly studied and a consensus has been reached on appropriate methods, critical drug concentrations that define resistance, and reliability and reproducibility of testing. Data on reproducibility and reliability of DST for the remaining second line drugs are either much more limited or have not been established, or the methodology for testing does not exist. Most importantly, correlation of DST results with clinical response to treatment has not yet been adequately established. Thus, a strain of TB showing resistance in *in vitro* DST could, in fact, turn out to be susceptible in the patient. The

prognostic relevance of *in vitro* resistance to drugs without an internationally accepted and standardized drug susceptibility test remains unclear and the current WHO (1997) recommendations disadvise to rely on such results in treatment guiding.

2 Methods

In the present study, the phenotypic and genotypic resistance of *M. Tuberculosis* strains of 19 patients from 2012 to 2013 was compared. In addition, six pan-susceptible strains were investigated with molecular-genetic tests.

2.1 Drug Susceptibility Testing (DST)

For phenotypic confirmation, a conventional 1 % proportion phenotypic drug susceptibility testing (DST) on Lowenstein Jensen (LJ) medium was used (Canetti et al. 1969). All samples were tested for resistance to isoniazid (INH) (0.2 µg/mL), rifampin (RIF) (40 µg/mL), streptomycin (STM) (4 µg/mL), ethambutol (EMB) (2 µg/mL), kanamycin (KMC) (30 µg/mL), amikacin (AMI) (30 µg/mL), capreomycin (CPM) (10 µg/mL), moxifloxacin (MXF) (2 µg/mL), ethionamide (ETA) (40 µg/mL), and cycloserine (CSR) (30 g/mL).

2.2 Genotypic Testing

For genotypic confirmation, the commercial kits MTBDRplus and MTBDRsl (Hain Lifescience; Nehren, Germany) were used. In the first step, all phenotypically confirmed resistant strains were evaluated with MTBDRplus test, which is designed for detection of INH and RIF resistance. The identification of RIF resistance is enabled by the detection of most significant associated mutations of rpoB gene. For detection of INH resistance, the katG gene and the promoter region of the inhA gene were examined.

Subsequently, all strains confirmed as MDR-TB were evaluated with MTBDRsl test, which is designed for detection of EMB, aminoglycosides, cyclic peptides (AG/CP), and fluoroquinolones (FLQ) resistance. The identification of FLQ resistance is enabled by the detection of the most significant associated mutations of the gyrA gene. For detection of AG/CP resistance, the 16S rRNA gene (rrs) and for EMB resistance, the embB gene were examined.

In both steps, PCR and hybridization were performed according to the manufacturer's instructions. For amplification, 35 μL of primer nucleotide mix (PNM), 5 μL of 10 × amplification buffer (Qiagen, Valencia, CA), 1.2 μL of 2.5 M MgCl$_2$ (Qiagen, Valencia, CA), 5 μL of DNA (15 ng/μL), and water were added to a final volume of 50 μL. PCR-amplified with biotinylated primers was performed in a thermal cycler with the following conditions: denaturation 95 °C/15 min, initial denaturation 95 °C/30 s, annealing 58 °C/2 min (10 cycles), denaturation 95 °C/25 s, annealing 53 °C/40 s, extension 70 °C/40 s (30 cycles), and final extension 70 °C/8 min. Reverse hybridization was performed as per manufacturer's instructions.

After successful hybridization, each zone in DNA strip was evaluated. TUB zone hybridizes with amplicons, generated from all members of *M. tuberculosis* complex. The locus control zones enhance the expression of linked genes (rpoB, katG, and inhA in MDBDRplus and gyrA, rrs, and embB in MTBDRsl). Wild-type probes comprise the most important resistant regions of the respective genes and Mutation-probes detect some of the most common resistance-mediating mutations.

3 Results

Sixteen out of the 19 previously phenotypically confirmed INH resistant strains were confirmed as being resistant using the PCR methods. All of the 10 phenotypically confirmed RIF resistant strains were confirmed as being resistant also genotypically. In none of the INH or RIF susceptible strains was resistance detected genotypically (Table 1).

Only nine strains, those previously confirmed as MDR-TB, were tested with MTB-DRsl test. Among them, seven were phenotypically confirmed as ETB susceptible and two as resistant. The molecular testing showed, however, resistance in another two strains. Six strains were phenotypically confirmed as susceptible to AG/CP and three were resistant. The resistance was confirmed genotypically only in two of them. Finally, all nine strains were phenotypically confirmed susceptible to moxifloxacin, but in six of them resistance was detected by molecular method (Table 2).

4 Discussion

The purpose of this study was to compare conventional methods, including cultivation and drug susceptibility tests, with modern molecular-genetic testing in the diagnosis of drug resistant form of tuberculosis. Conventional methods require a long time to be performed. The average time of cultivation *M. tuberculosis* is 42 days; subsequent DST takes another 21–25 days. During that time patients can be treated inappropriately and resistant strains may continue to spread.

Using molecular tests we were able to confirm RIF resistance in all previously phenotypically found resistant strains. In the majority of strains, 8 out of the 10, resistance was detected in codons at position 513–519 of rpoB gene. INH resistance was confirmed in 16 out of the 19 previously confirmed resistant strains. The most common resistance locus was detected in codons at position 315 of katG gene (13 out of the 16). In three cases resistance was detected only at the nucleic acid position 15 in the inhA promoter region. Five strains demonstrated resistance in both genes. Undetected resistance in three of the previously phenotypically confirmed INH resistant strains could be due to mutations occurring in other genes, such as aphC, kasA, or oxyR.

ETB resistance was detected in the embB gene in both previously confirmed resistant

Table 1 Comparison of phenotypic and genotypic determination of drug resistance

| Isolates code | Phenotypic pattern | | Genotypic pattern | |
	INH	RIF	INH	RIF
5/2012	R	R	*katG*, WT, MUT 1	*rpoB*, WT 3,4, MUT
10/2012	R	S	**S**	**S**
13/2012	R	R	*katG*, WT, MUT 1, *inhA* WT 1, MUT 1	*rpoB*, WT 3,4, MUT 1
127/2012	R	S	**S**	**S**
133/2012	R	S	*inhA* WT 1, MUT 1	**S**
147/2012	R	S	*katG* WT 1, MUT 1, *inhA* WT 1, MUT 1	**S**
207/2012	R	S	*katG* WT 1, MUT 1, *inhA* WT 1, MUT 1	**S**
249/2012	S	S	**S**	**S**
327/212	S	S	**S**	**S**
337/2012	S	S	**S**	**S**
16/2013	S	S	**S**	**S**
18/2013	R	R	*inhA* WT 1, MUT 1	*rpoB* WT 8, MUT 3
44/2013	R	S	**S**	**S**
62/2013	R	R	*katG*, WT, MUT 1	*rpoB*, WT 3,4, MUT 1
68/2013	R	S	*katG*, WT, MUT 1	**S**
112/2013	R	S	*katG*, WT, MUT 1	**S**
120/2013	R	R	*katG*, WT, MUT 1	*rpoB, WT 8, MUT 3*
175/2013	R	R	*katG*, WT, MUT 1	*rpoB*, WT 3,4, MUT 1
186/2013	R	S	*inhA* WT 1, MUT 1	**S**
188/2013	R	R	*katG* WT 1, MUT 1, *inhA* WT 1, MUT 1	*rpoB*, WT 3,4, MUT 1
220/2013	R	R	*katG*, WT, MUT 1	*rpoB*, WT 3,4, MUT 1
221/2013	R	R	*katG*, WT, MUT 1	*rpoB*, WT 3,4, MUT 1
222/2013	R	R	*katG* WT 1, MUT 1, *inhA* WT 1, MUT 1	*rpoB*, WT 3,4, MUT 1

INH isoniazid, *RIF* rifampin

Table 2 Comparison of phenotypic and genotypic determination of drug resistance (ETB, AG/CP, FLQ)

| Isolates code | Phenotypic pattern | | | Genotypic pattern | | |
	ETB	AG/CP	FLQ	ETB	AG/CP	FLQ
5/2012	**S**	**S**	**S**	**S**	**S**	**S**
13/2012	**S**	**R**	**S**	**S**	*rrs*, WT 1, MUT1	*gyrA*, WT3, MUT1
18/2013	**S**	**R**	**S**	**S**	*rrs*, WT 2	*gyrA*, WT3, MUT 3C
62/2013	**S**	**S**	**S**	*embB* WT1, MUT 1A	**S**	*gyrA*, WT3, MUT 3C
120/2013	**S**	**S**	**S**	**S**	**S**	**S**
175/2013	**R**	**S**	**S**	*embB* WT1, MUT 1A	**S**	*gyrA*, WT3, MUT 3C
186/2013	**S**	**S**	**S**	**S**	**S**	*gyrA*, WT3, MUT 3C
220/2013	**R**	**S**	**S**	*embB* WT1, MUT 1A	**S**	*gyrA*, WT3, MUT 3C
222/2013	**S**	**S**	**S**	*embB* WT1, MUT 1A	**S**	**S**

strains. In addition, resistance was detected also in the other two strains, which were phenotypically confirmed as susceptible. This may have been caused by the previously described limitation of conventional drug susceptibility testing for EMB. Kim (2005) has reported that 90 % of probably EMB-susceptible strains and 30–50 % of probably EMB-resistant strains are inhibited at the critical concentration of 2 µg/mL on LJ solid medium. Park et al. (2012), on the other hand, has reported that mutation in the emB gene does not affect ETB resistance.

Resistance to aminoglycosides or cyclic peptides was phenotypically confirmed in two of our strains, which was in accord with the subsequently done molecular testing.

5 Conclusions

The molecular-genetic methods provide prompt, accurate, and of high quality diagnosis of tuberculosis. Although there are some discrepancies in genotypically and fenotypically confirmed resistance, predominantly for INH, ETB, and FLQ, the molecular methods should be available in the National Reference Laboratory for the patients who are suspected of having resistant TB. Nevertheless, drug susceptibility testing remains the gold standard for the final diagnosis of drug resistant tuberculosis.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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Does Customer Information Fulfill MEDDEV Criteria in Cases of Product Problems of *In Vitro* Diagnostics for Infection Testing?

Jürgen Hannig and Rüdiger Siekmeier

Abstract

The European Directive 98/79/EC on *in vitro* diagnostics (IVD) regulates marketing and post market surveillance of IVD in the European Economic Area. In cases of incidents and field safety corrective actions (FSCA) manufacturers have to inform responsible competent authority (CA) and public by field safety notices (FSN). We analyzed FSCA and FSN of IVD for infection testing (culture media, reagents, kits, control materials, as well as culture-based analyzers and their general consumables) published by the Federal Institute for Drugs and Medical Devices (BfArM) in Bonn, Germany in 2005–2012 in regard to the European Regulatory Framework of Medical Devices (MEDDEV). One hundred and sixty-nine FSCA were published and German and English FSN were found in 157 and 154 cases, respectively. FSN were clearly characterized as FSN in 110 German and 134 English cases and product names were provided in 157 and 154 cases, respectively. Lot numbers and other information for product characterization were available in 146 and 137 cases, respectively. The information regarding FSCA and product malfunction was provided in 157 and 151 and 144 and 136 cases and that regarding the product related risks with continued use of affected IVD in 116 and 116 cases, respectively. In 156 German and 152 English cases, manufacturers provided the information for risk mitigation, including retesting in 69 and 75 cases, respectively. Requests to pass FSN to persons needing awareness were found in 108 and 87 cases, and contact data were provided in 127 and 131 cases, respectively. We conclude that most FSN fulfilled the MEDDEV criteria. However, type and content of FSN should be improved to ensure a better mitigation of risks due to product failure.

J. Hannig (✉)
c/o Prof. Dr. Schweim
Drug Regulatory Affairs, Pharmaceutical Institute,
University Bonn, An der Immenburg 4, Bonn 53121,
Germany
e-mail: juergenH79@googlemail.com

R. Siekmeier
Drug Regulatory Affairs, Pharmaceutical Institute,
University Bonn, An der Immenburg 4, Bonn 53121,
Germany

1   Introduction

Directive 98/79/EC (1998) regulates conformity assessment, marketing, and post- marketing surveillance of *in vitro* diagnostic medical devices (IVD) in Europe. The substance of the Directive has been implemented in Germany by means of the second Amendment of the German Law on Medical Devices (MPG, Medizinproduktegesetz) on January 1, 2002 (Medizinproduktegesetz 2002), which was subject of several revisions since. The latter has been flanked by the Ordinance on the Medical Devices Vigilance System (MPSV, Medizinproduktesicherheitsplanverordnung) from June 24, 2002 (Verordnung über die Erfassung, Bewertung und Abwehr von Risiken bei Medizinprodukten 2002), which was also revised later on. In brief, the manufacturers shall be obliged to systematically review the experience gained from devices on the market, to implement corrective actions, where necessary, and to report incidents and recalls to the responsible competent authority (CA). In Germany, according to the Ordinance on the Medical Devices Vigilance System, also professional operators and users have to report incidents that they observe in using the products to the CA (Guidelines on a Medical Devices Vigilance System 2013; Medical Devices Post Market Surveillance 2009; Bornhak et al. 2002). The same obligation applies to pharmacies and other retail traders if incidents related to over-the-counter products sold to lay people come to their knowledge. The Federal Institute for Drugs and Medical Devices (BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte) and the Paul-Ehrlich-Institute (PEI) are responsible for registration and examination of issues related to IVD. The latter is responsible only for a few IVD for infection testing and immune hematological diagnostics as well as tissue-typing as specified in Annex II of Directive 98/79/EC (Siekmeier et al. 2008, 2009a, 2010; Bornhak et al. 2002; Directive 98/79/EC 1998).

The task of CA is to characterize the risk (in terms of the probability of occurrence of harm and the severity of it) and to assess its acceptability. In case of unacceptable risks, a necessary corrective action is to be determined. If manufacturers have already taken measures in their own responsibility, the CA has to take a decision on whether or not these are adequate. Any necessary field safety corrective action (FSCA) performed by the manufacturers must be properly communicated to the customers and users. In Germany, this is typically done by contemporary sending of field safety notes (FSN); the FSN must also be sent to the BfArM for information and publication on the homepage. In cases of FSCA brief information regarding the affected product and the corresponding FSN is also published on the BfArM homepage.

As CE-marked devices (declaration of conformity with EU medical devices) in principle enjoy free movement in the entire European Economic Area (EEA), there is a need for information to be exchanged between CAs, in particular when a field corrective action is to be taken. The Directive therefore requires that the European CAs inform each other and the European Commission of cases that lead to corrective actions. Having been informed through a national competent authority report (NCAR), all CAs can then monitor the corrective action in their area of responsibility (if this is deemed to be necessary) and also consider whether similar products of other manufacturers may also be affected by the observed problem.

Up to now only few data regarding the experience on market surveillance have been published demonstrating differences within the very heterogeneous group of IVD in respect to the source of notification to the CA, the cause of product failure, and the measures taken by the manufacturers for mitigation of product related risk (Siekmeier and Wetzel 2013a, b; Siekmeier et al. 2008, 2009a, 2010; Halbauer et al. 2009; Siekmeier

Table 1 Required content of field safety notices (FSN) according the guideline MEDDEV 2.12-1 rev 8 (Guidelines on a Medical Devices Vigilance System 2013)

1. A clear title, with 'Urgent field safety notice' followed by the commercial name of the affected product, an FSCA-identifier (e.g., date) and the type of action
2. Specific details to enable the affected product to be easily identified e.g., type of device, model name and number, batch/lot or serial numbers of affected devices and part or order number
3. A factual statement explaining the reasons for the FSCA, including description of the device deficiency or malfunction, clarification of the potential hazard associated with the continued use of the device and the associated risk to the patient, user or other person and any possible risks to patients associated with previous use of affected devices

4. Advice on actions to be taken by the user. Include as appropriate
    Identifying and quarantining the device
    Method of recovery, disposal or modification of device
    Recommended review of patients previous results or patient follow up, e.g., implants, IVD
    Timelines

5. A request to pass the field safety notice to all those who need to be aware of it within the organization and to maintain awareness over an appropriate defined period
6. If relevant, a request for the details of any affected devices that have been transferred to other organizations, to be given to the manufacturer and for a copy of the field safety notice to be passed on to the organization to which the device has been transferred
7. If relevant, a request that the recipient of the field safety notice alerts other organizations to which incorrect test results from the use of the devices have been sent. For example failure of diagnostic tests
8. Confirmation that the relevant National Competent Authorities have been advised of the FSCA

9. Comments and descriptions should be omitted, which attempt to
    (a) Serve to play down the level of risk in an inappropriate manner
    (b) Advertise products or services

10. Contact point for customers how and when to reach the designated person. An acknowledgment form for the receiver might also be included (especially useful for manufacturer's control purposes)

and Lütz 2007a, b; Spitzenberger et al. 2007). Only in one of these publications, the FSN sent by the manufacturers in case of an FSCA was subject to the investigation (Siekmeier et al. 2010). However, that study did not investigate in detail if the FSN complies with the MEDDEV criteria (Table 1). Therefore, aim of the present study was to analyse the field safety notices and field safety corrective actions concerning the *in vitro* diagnostics for infection testing published by the BfArM in respect to the MEDDEV criteria 2.12-1 rev 8.

2 Methods

All notifications on IVD published by the BfArM in 2005–2012 were included in the study, as there is no relevant number of publications of FSCA on the BfArM homepage before 2005. Detailed analysis was made for IVD for infection testing (culture media, reagents, kits, control materials, and culture-based analyzers, e.g., for strain differentiation and susceptibility testing) and their general consumables (e.g., buffers, pipettes, or cleaning reagents). Analyzers based on molecular biological or immunological means were excluded, as they are often used for other analytical parameters (e.g., genetic testing, oncology, or clinical chemistry). The included FSCA were analyzed in respect to the criteria of MEDDEV 2.12-1 rev 8 (Table 1).

3 Results

3.1 Number of Reports and FSCA

BfArM received a total of 3898 reports regarding IVD between 1999 and 2012, from which 3,452 were received since the beginning of 2005. Publication of FSCA and FSN by BfArM started as of the end of 2004, and there were 1,257 FSCA published for IVD within the study period

Table 2 Number of *in vitro* diagnostics (IVD) related notifications to the BfArM, and field safety corrective actions (FSCA) published by BfArM in 1999–2012 (BfArM homepage 2013; Siekmeier and Wetzel 2013b; Siekmeier et al. 2008, 2009b; Siekmeier and Lütz 2007b)

Year	Number of notifications	FSCA published
1999	13	–
2000	21	–
2001	33	–
2002	58	–
2003	121	–
2004	200	–
2005	207	135
2006	235	116
2007	583	150
2008	506	143
2009	392	149
2010	482	180
2011	474	194
2012	573	190
Sum	3,898	1,257

The number of notification was 3,452 since the beginning of 2005

01. 01. 2005 – 31. 12. 2012. From these, 169 affected culture media, reagents, kits and control materials, and culture-based analyzers and their general consumables for infection diagnostics. The number of annual reports regarding IVD and the corresponding FSCA strongly increased within this time (Table 2).

3.2 Fulfilment of MEDDEV Criteria

German and English FSN were found in 157 and 154 cases, respectively. FSN were clearly characterized as FSN in 110 and 134 cases and the names of the affected products were provided in 157 German and 154 English cases. Lot numbers or other information for product characterization was available in 146 and 137 cases, respectively. Detailed information regarding the FSCA was found in 157 and 151 and that regarding product malfunction in 144 and 136 cases, respectively. More detailed analysis was made independent from the language in all FSN. Most frequently product malfunction was due to a fault in the raw material or in the construction of a product (32 cases). Stability issues or a decrease in sensitivity were also a serious problem (27 cases). Impairment or variations in product performance, faulty standardization, or an error in the specification were found in 20 cases, and in 15 cases a contamination was the reason for product malfunction. Erroneous isolation, identification, or a bacterial resistance were found in 13 cases and in 4 cases a slower growth pattern of bacterial strains was identified. Shipping of unsuitable, incorrectly, or mislabeled products played only a minor role (9 cases), and shipping or correction of erroneous and revised package inserts was the problem in 7 cases. There were 12 cases with malfunctions in software and wrongly installed software or data transmission. No precise indication of a product failure was found in 18 cases and in 1 case there was no product failure (change of the package insert to the latest status).

Information on the product related risks with continued use of affected IVD was provided in 116 German and 116 English cases. In most of these cases, manufacturers informed customers only about erroneous results (61 and 58, respectively). A more precise statement whether the result is falsely positive or falsely negative was found in 22 and 23, and 22 and 23 German/English cases, respectively. In further 2 German and 1 English case, manufacturers stated that the results were at the lower or higher end of product specification. In one more case, German and English FSN informed customers about the risk for either lost or mixed-up results between samples sharing bottles with duplicate bar codes. No analytical error, but information concerning the risk of poisoning, contamination, injury, or infection was found in 4 German and 4 English cases. However, in 5 and 3 cases, respectively, the manufacturer informed the affected customers about a low or no risk in continued use of the product.

In 156 German and 152 English cases, manufacturers provided the information for a mitigation of product related risks. These were instructions for risk mitigation (e.g., halt of use, discard of the product, or a request for replacement) in 102 and 102 cases, respectively. In 11 German and 10 English cases, customers

Table 3 Compliance of field safety notices to the guideline criteria of MEDDEV 2.12-1 rev 8. A total number of FSCA was 169

	German FSN	English FSN
Number of FSN	157	154
FSN is clearly identified for users	110	134
Declaration of the product name in the FSN	157	154
Declaration of the Lot-No. or other attributes for product identification	146	137
Information regarding the reason of FSCA	157	151
Description of the device malfunction in the FSN	144	136
Clarification of the potential hazard associated with use	116	116
Directions for mitigation of product related risks	156	152
Resulting in control/retest of prior obtained results	69	75
Request to pass the FSN to other persons who need to be aware	108	87
Comments playing down product risks or advertising the product	34	31
Declaration of a contact person or phone number	127	131
Confirmation that the relevant national CA has been informed	39	37
Acknowledgement form for the receiver included in the FSN	132	111

FSN - field safety notices; *FSCA* - field safety corrective actions, *CA* competent authority

were advised to confirm all future results by an alternative analytical method and in 43 and 40 cases, respectively, additional safety or procedural steps were needed to be implemented. Recommendations for a control of previous results or retesting were found in 69 and 75 cases, respectively.

Requests to pass the FSN to persons or organizations needing awareness were found in 108 German and 87 English cases. Comments playing down the product related risks or advertising the product were found in 34 and 31 FSN and contact data were provided in 127 and 131 cases, respectively. Finally, confirmation that a CA was informed was found in 39 German and 37 English cases and in 132 and 111 cases, respectively, a customer confirmation form was included (Table 3).

Comparison of the German and English FSNs not only demonstrated differences in the number of MEDDEV criteria fulfilled, but also differences in the FSN for the affected products (product or Lot-No.), contact data of the manufacturers, and the name of the informed CA. These differences were likely caused by the distribution of different products/lots, different national subsidiaries of the manufacturers, and different responsible CAs in various countries and assumed to be not critical.

However, there were also cases of differences in the measures to be taken by the users (e.g., due to differences in customer education and sample handling and laboratory organization in different countries) and in requests for passing the FSN to other persons and organizations (e.g., due to different types of laboratory organization in different countries) which may be critical and therefore should be subject to thorough evaluation.

4 Discussion

In 1999–2012, BfArM received a total of 3,898 reports regarding IVD from which 3,452 were received since the beginning of 2005. In the first years, the number of notification was very low, but later there was a rapid increase suggesting that the European system for market surveillance functioned well, although there was an unknown rate of underreporting (Siekmeier and Wetzel 2013a, b; Siekmeier et al. 2008, 2009a, 2010; Siekmeier and Lütz 2007a, b). In 2005–2012, 1,257 FSCA were published for IVD on the homepage of the BfArM resembling 36.4 % of all notifications related to IVD within this time (BfArM homepage 2013). This proportion is much lower than that of corrective actions

reported before for other types of IVD for professional use, e.g., analyzers and their general consumables for diagnostics of infectious diseases (89.1 % and 94.4 %, respectively) (Siekmeier et al. 2009a), reagents for diagnostics of infectious diseases (81.1 %) (Siekmeier et al. 2008), reagents and analyzers for coagulation testing (78.1 % and 90.0 %, respectively), reagents for diagnostics of tumor diseases (76.2 %), and reagents and analyzers for therapeutic drug monitoring (85.2 % and 90.9 %, respectively), but much higher than the proportion of corrective actions reported for most lay use of IVD (mostly systems for self-testing of blood glucose (24.7 %), blood coagulation (69.2 %), pregnancy (8.0 %)) (Siekmeier and Lütz 2007a, b). In those studies, corrective actions not affecting the German market were not evaluated. The difference between various types of professional and lay use IVD is caused by a high proportion of confirmed product failures in professional use IVD and a low proportion of confirmed product failures in lay use IVD (except for IVD for self-testing of coagulation). The proportion of 36.4 % (1,257 FSCA out of the 3,452 notifications) in the present study concerns all IVD, i.e., those for the professional and lay use. Therefore, it is likely, that this result is affected by a high number of notifications related to the lay use IVD, at least at the beginning of the observation period 2005–2012 (Siekmeier et al. 2009b). However, it should be considered that only FSCA affecting the market (e.g., recalls or customer information) are subject to publication by the BfArM, whereas other corrective actions (e.g., preventive measures in production and quality control or changes of raw materials) are not. Moreover, only FSCA affecting the German market are subject of publication also resulting in a lower rate of corrective actions compared with prior publications.

A total number of FSCA included 169 cases related to IVD for infection diagnostics (tests, reagents, calibrators, culture media, and culture-based analyzers and their general consumables), making up 13.4 % of all IVD-related FSCA published by the BfArM in 2005–2012. This number demonstrates the importance of this product subgroup. However, it should be noted that a relevant number of IVD for infection diagnostics are missing since they are not included as IVD for infection testing, immune hematological testing, and tissue typing listed in Annex II parts A and B of Directive 98/79/EC. Market surveillance for these IVD in Germany is in the responsibility of the Paul-Ehrlich-Institute (Siekmeier et al. 2008, 2009a, 2010; Halbauer et al. 2009; Spitzenberger et al. 2007; Bornhak et al. 2002; Directive 98/79/EC 1998) and FSCA for these products are not published. Therefore, FSCA related to tests, reagents, calibrators and control materials for diagnostics of human immune deficiency virus I and II (HIV-I, HIV-II), human T-cell leukemia virus I and II (HTLV-I, HTLV-II), hepatitis B, C, and D, cytomegaly, rubella, chlamydia and toxoplasmosis were not included in our study (81 notifications regarding these products to PEI between 2005 and 2007) (Halbauer et al. 2009).

Failures in IVD may cause a direct harm to users (e.g., infection) and an indirect harm to patients (e.g., due to delayed diagnostics, erroneous results, and unnecessary treatment). In addition, failure in IVD for infection testing may also cause a risk for infection of third persons (e.g., relatives of patients after delayed or erroneous test results). These risks once more underline the importance of IVD for infection testing and the importance of FSN in cases of product failure. Siekmeier et al. (2010) have investigated FSN in FSCA of IVD for infection testing (tests, reagents, calibrators, control materials and analyzers and their general consumables) and found that FSN of sufficient quality were available in most of the FSCA, but there was a substantial delay between sending out a FSN notification and receiving it by the BfArM, resulting in a delayed publication (Siekmeier et al. 2010). The present study is based on a larger number of cases and performs a more detailed analysis of FSN in respect to the MEDDEV 2.12-1 rev 8, whereas data regarding the time delay of publication were not available. German and English FSN were found in 157 and 154 out of 169 FSCA, respectively. Most likely,

the differences between the number of FSCA and FSN as well as the number of German and English FSN are caused by the number of missing FSN in cases where no FSN has been sent to the BfArM (e.g., information of few customers by phone only). Most FSN were clearly characterized as FSN and included the names of the affected products according to requirements of the MEDDEV 2.12-1 rev 8. Lot numbers and other information for product identification were also provided by most FSN, but the number of FSN complying with the MEDDEV criteria was smaller, even though distinct product identification is essential in cases of FSCA. Manufacturers, in the majority of FSN, provided sufficient information regarding FSCA and product malfunction. However, deviations from the MEDDEV requirements were observed and should be avoided, as clear descriptions serve as a basis for an understanding the FSCA and measures to be performed by users. Information for a mitigation of product related risks was found in 156 out of the 157 German and 152 out of the 154 English FSN. Although these numbers are high, one should consider that this information is essential in an FSN. A critical non-compliance to the MEDDEV requirement was found regarding the clarification of a potential hazard associated with continued use of an affected product. This information was provided in 116 and 116 out of the 157 and 154 German and English FSN, respectively, which demonstrates the lack of this information in about one third of the FSN analyzed in the present study. This is in accord with the results of our previous study concerning the point of care tests and analyzers for blood gas content and electrolytes, where 24 and 24 out of the 32 and 31 German and English FSN, respectively, provided this information (Hannig and Siekmeier 2013).

In 69 German and 75 English FSN, manufacturers recommended a control or retesting of results, which underlines the importance of the IVD for infection testing and the risk caused by a product failure. Requests to pass the FSN needing awareness to the involved persons in the organization were found in 108 German and 87 English FSN. However, these requests are necessary to ensure the spread of information because different users and split responsibilities may exist within organizations. Contact data were also provided in 127 German and 131 English FSN only, which may also be critical, as users may have queries regarding the FSCA. Although the comments playing down the product related risks or advertising the product were found only in a minor number of FSN (34 German/31 English), this is important because FSNs are issued in cases of product failure and product related risks and such comments foil the intention of the FSN. Customer confirmation forms were included in the majority of FSN (132 German/111 English). However, these should be included in the FSN because they are useful for manufacturers' control purposes.

Comparison of German and English FSN not only demonstrates differences in the number of observed MEDDEV criteria, but also differences in the affected products (product or Lot-No.), contact data of the manufacturer, and the name of the informed CA. These differences are most likely caused by distribution of different products/lots, different national subsidiaries of manufacturers, and different responsible CAs, and assumed to be not critical. However, there were also cases of differences of the measures to be taken by users (e.g., due to differences in customer education and sample handling and laboratory organization in different countries) and in requests for passing the FSN to other persons and organizations, which may be critical and therefore should be subject to a thorough evaluation.

In summary, a strong increase in notifications to the BfArM and a high number of published FSCA demonstrate that the European surveillance system for IVD is an established tool for ensuring the safety of these products even though there is a need for some optimization. For example, there are currently no data available regarding FSN for FSCA affecting the German market in cases of high risk IVD for infection diagnostics listed in Annex II of Directive

98/79/EC. Furthermore, as the distribution of FSN in cases of FSCA is an important means for a reduction of product related risks of IVD already in the market, the type and content of published FSN should comply with the requirements of the guidelines MEDDEV 2.12-1 rev 8.

Conflicts of Interest The authors report no conflicts of interest in relation to this article.

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Sodium and Copeptin Levels in Children with Community Acquired Pneumonia

August Wrotek, Teresa Jackowska, and Katarzyna Pawlik

Abstract

Copeptin has been associated with the severity of pneumonia and its complications. This study was designed to assess the usefulness of copeptin measurement in children with community-acquired pneumonia (CAP) and copeptin's relation with disease severity and sodium equilibrium. The study encompassed 311 patients (227 with pneumonia and 84 healthy controls) aged 8 days–18 years. Clinical findings and inflammatory markers were used to predict the disease severity. We found that the level of copeptin was significantly higher in patients with CAP (median 0.88 ng/mL) *vs.* healthy children (0.33 ng/mL; $p < 0.01$). ROC analysis showed a high AUC value (0.87) and the cut-off point for plasma copeptin level was 0.44 ng/mL, with a high sensitivity (89 %) and specificity (73 %) in recognizing pneumonia. Patients with higher copeptin concentrations were at higher risk of hyponatremia (OR 2.43). Yet there was only a weak reverse correlation between the sodium and the copeptin concentrations (Spearmann's rank coefficient $= -0.19$). The levels of copeptin were higher in hyponatremic patients (0.83 ng/mL) *vs.* normonatremic patients (0.69 ng/mL; $p = 0.02$). Copeptin elevation did not reflect the CAP severity measured with traditionally used methods. In conclusion, copeptin elevation is a promising marker of pneumonia, but it reflects neither the disease severity nor sodium concentration.

A. Wrotek, T. Jackowska (✉), and K. Pawlik
Department of Pediatrics, Medical Center of Postgraduate Education, 99/103 Marymoncka St.,
Warsaw 01-813, Poland

Department of Pediatrics, Bielanski Hospital,
Warsaw, Poland
e-mail: tjackowska@cmkp.edu.pl

1 Introduction

Pneumonia in children under 5 years of age is the major cause of mortality and most of the cases involve community-acquired pneumonia (CAP) (Rudan et al. 2008, 2013). Worldwide estimates report about one million deaths every year in this

age group, excluding neonates (Liu et al. 2012). Although the highest incidence and mortality due to pneumonia are observed in low- and middle-income countries, pneumonia still has a huge impact on developed regions. There are approximately 2.6 million cases of pneumonia in children aged less than 5, which is responsible for 3,000 deaths each year. Approximately, 50–60 % of such children require hospitalization (around 1.5 million hospitalizations in high-income countries) because of CAP (Madhi et al. 2013). The incidence of pneumonia in Europe varies between 0.015 and 0.060 episodes is about 0.22 episodes per child-year (Rudan et al. 2013). In adults with pneumonia, the level of copeptin is shown to predict clinical deterioration and mortality (Kolditz et al. 2012; Christ-Crain et al. 2008). Yet only single research has been conducted in pediatric patients, in whom a correlation between the increased copeptin level and the presence of complications, especially pleural effusion, is reported (Du et al. 2013).

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is commonly observed in patients with pneumonia and is the main cause of hyponatremia (Dhawan et al. 1992; Singhi and Dhawan 1992). In humans, the main antidiuretic hormone (ADH) is arginine vasopressin (AVP). SIADH develops in pathological conditions, e.g., inflammatory infiltration in lung tissue, which increases the AVP synthesis. The measurement of AVP in the serum is difficult due to its low biological stability (Nigro et al. 2011), but there is a way to assess its concentrations from the copeptin level. Copeptin derives from pre-provasopressin which consists of a signal peptide, AVP, neurophysin II, and copeptin and is co-secreted with AVP in an equimolar ratio (Morgenthaler et al. 2007). Copeptin, in contrast to AVP, may be easily and reliably measured in the serum. The copeptin level directly reflect that of AVP (Szinnai et al. 2007; Jochberger et al. 2006), and thus may be used as a surrogate of AVP.

Inappropriate AVP secretion leads to hyponatremia in the majority of CAP cases complicated by SIADH. Hyponatremia is the most frequent electrolyte disorder and affects up to 3 % of hospitalized patients; a low sodium level in patients with pneumonia is much more frequent and has been observed in 27–45 % of children with CAP (Don et al. 2008; Singhi and Dhawan 1992). Although most cases of hyponatremia are mild, this symptom has been related to the severity of CAP. Hyponatremic patients tend to have increased serum inflammatory markers, higher body temperature at admission, longer hospital stay (Wrotek and Jackowska 2013; Sakellaropoulou et al. 2010; Don et al. 2008), or even a higher frequency of complications and mortality (Singhi and Dhawan 1992). Moreover, hyponatremia does not depend on the etiology of pneumonia or the type of radiological consolidation (Don et al. 2008), and thus may be a promising and widely available marker of CAP severity. Copeptin, which is expected to be increased in CAP patients, especially those with SIADH and hyponatremia, may be a possible biomarker of pneumonia and its severity. The measurement of copeptin in hyponatremic patients may expand the body of knowledge about the sodium-copeptin association and its possible role in the pathogenesis of hyponatremia.

This study was designed to assess the usefulness of copeptin measurement in differentiating patients with pneumonia from healthy children and the association of elevated copeptin level with the severity of CAP. An additional goal of the study was to investigate the sodium-copeptin association in CAP.

2   Methods

The research and its protocol have been approved by a local Ethics Committee and the study has been performed in accord with the Declaration of Helsinki for Human Experimentation. This prospective study on hyponatremia-copeptin-pneumonia was conducted at the Department of Pediatrics of the Medical Center of Postgraduate Education in Warsaw, Poland. Initially, 477 children hospitalized between January 2011 and March 2013 were diagnosed to have community-acquired pneumonia, but only 412 were eligible

for the study. Patients described herein were previously reported in a paper on hyponatremia in children hospitalized due to pneumonia; therefore the inclusion and exclusion criteria remained identical with those described previously (Wrotek and Jackowska 2013).

Concerning the control group, the main inclusion criterion was the lack of triggers and conditions that might have an impact on the copeptin level or water balance according to the current knowledge; the children included into the control group were hospitalized due to suspicion of a disease that was finally excluded (e.g., intoxication or urinary tract infection), probable psychological background of complaints (chest pain or stomachache with no diagnosed organic cause), or having checkups after the treatment of cured disease (e.g., anemia). Moreover, to exclude patients with asymptomatic or chronic inflammatory disease, only children with high-sensitive C-reactive protein (hsCRP) concentration within the normal range met the inclusion criteria. If there were any doubts on the possible influence of a given disease on the copeptin or sodium level, the children were excluded from the control group. The exclusion criteria also comprised any severe disease or condition, even previously diagnosed (like proliferative disease or diabetes mellitus) and medications altering the sodium or copeptin levels (e.g., diuretics).

In each case, sodium level was measured in the specimen that was collected on admission. The specimens were stored at $-70\,^{\circ}\mathrm{C}$ and subsequently used for copeptin measurement. Out of the 412 (206 male, 206 female) patients with CAP, 227 children (114 female, 113 male), aged 8 days to 18 years (median 39 months) were chosen at random and had their copeptin levels measured. The control group consisted of 84 (44 female, 40 male) healthy controls aged 22 days to 18 years (median 46 months). In total, copeptin was measured in 311 patients: 227 with pneumonia and 84 healthy controls.

To assess the CAP severity, we used clinical findings (body temperature at admission, defeverscence time, heart rate, breath rate, capillary blood saturation, length of antibiotic course, and length of hospital stay) as well as inflammatory markers (C-reactive protein – CRP, procalcitonin, white blood cell count, and neutrophil percentage). In accordance with physiological differences among the age groups, the breath frequency and heart rate were analyzed separately in children under and over 1 year of age. Likewise, the white blood cell count and the neutrophil percentage were analyzed separately in children under and over 4 years of age. Hyponatremia was defined as the serum Na^+ concentration under 136 mmol/L and the severity of hyponatremia was classified according to Ellison and Berl (2007) (mild hyponatremia: 131–135 mmol/L, moderate: 126–130 mmol/L, and severe: 125 mmol/L or lower).

Continuous variables were presented as means $\pm$SD for normally distributed data or as median and interquartile range (IQR) for skewed data. An unpaired t-test or the Mann-Whitney U test was used, respectively. To assess the association between copeptin and sodium levels and between copeptin and disease severity markers, Spearmann's rank correlation coefficient was used. A receiver operating characteristic (ROC) curve analysis was used to assess the usefulness of copeptin in predicting the outcome of pneumonia and to establish the cut-off points. In a multivariate logit model, continuous data were categorized on the basis of median values. The logistic regression results were shown as odds ratios (OR) with 95 % confidence interval (95 % CI). $P < 0.05$ was defined as showing statistical significance of differences. All analyses were performed using the commercial statistical package Statistica 10 (StatSoft).

3 Results

Copeptin concentration ranged from 0.014 to 14.520 ng/mL in the CAP group (one patient with the value exceeding the method's detectability was excluded from further analysis), and from 0.079 to 1.360 ng/mL in the healthy children. The median copeptin concentration in the CAP children was higher than that in the healthy children (0.88 ng/mL, IQR: 0.64–1.30 *vs.* 0.33 ng/mL, IQR: 0.20–0.46; $p < 0.01$)

Table 1 Copeptin concentrations (ng/mL) in patient groups

Median	LQ	UQ	Median	LQ	UQ	p
CAP (n = 226)			**Control (n = 84)**			
0.88	0.64	1.30	0.33	0.20	0.46	**<0.01**
Hyponatremia (n = 91)			**Non-hyponatremia (n = 209)**			
0.83	0.57	1.17	0.69	0.37	1.04	**0.019**

p-value (Mann-Whitney U test)
LQ lower quartile, *UQ* upper quartile

Table 2 Odds ratios (OR) with 95 % confidence intervals (CI) for copeptin-pneumonia and copeptin-hyponatremia associations
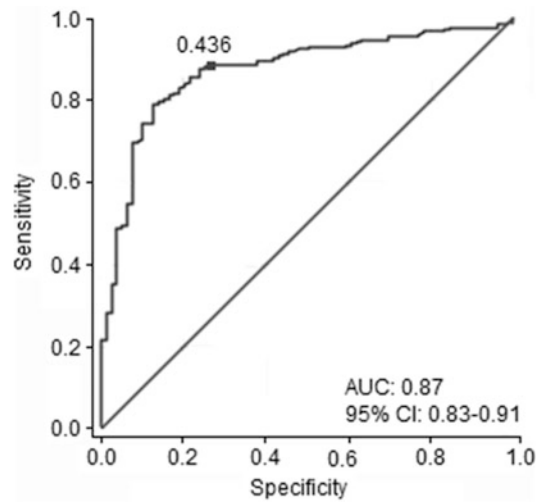
	OR	95 % CI	p
Copeptin – pneumonia	2.31	9.6–55.7	**<0.01**
Copeptin – hyponatremia	2.43	1.28–4.63	**<0.01**

Fig. 1 Plasma copeptin level – pneumonia in receiver operating characteristic curve analysis

Table 3 Spearmann's coefficient rank for sodium – copeptin correlation

Total	−0.19
Hyponatremia	NS
Non-hyponatremia	−0.16
CAP	NS
Control	NS

NS nonsignificant

(Table 1). Children with increased copeptin levels (i.e., above median) were at higher risk of developing pneumonia than those with lower copeptin levels (odds ratio 23.1, 95 % CI: 9.6–55.7, p < 0.01) (Table 2).

The ROC curve analysis showed the optimal cut-off point for plasma copeptin level in predicting pneumonia to be 0.436 ng/mL. The area under curve (AUC) value of 0.87 (95 % CI: 0.83–0.91) showed that copeptin may be treated as a possible biomarker of pneumonia, characterized by high sensitivity (89 %) and satisfying specificity (73 %), with a positive predictive value of 91 % and a negative predictive value of 69 % (Fig. 1).

The analysis of copeptin levels showed no correlation between copeptin and any of the markers of disease severity (laboratory or clinical). Patients with increased copeptin concentration (above median) tended to have higher body temperature (OR 1.9, 95 % CI: 1.1–3.3, p = 0.02), but there was no correlation between copeptin and body temperature in Spearmann's correlation rank or in ROC analysis.

The copeptin level was higher in the children with lowered sodium levels than in those with normal sodium level (0.83 ng/mL, IQR: 0.57–1.17 *vs.* 0.69 ng/mL, IQR: 0.37–1.04; p = 0.019) (Table 1). Multivariate logit analysis showed that patients with higher copeptin concentration (above median) were at over twofold higher risk of developing hyponatremia (OR 2.43, 95 % CI: 1.28–4.63, p < 0.01), although ROC analysis showed no acceptable cut-off point value.

There was a weak reverse correlation between the sodium and copeptin concentrations (Spearmann's rank coefficient = −0.19), but it was relevant only in the normonatremic patient subgroup (Spearmann's rank coefficient = −0.16), while it was insignificant in the children with lowered sodium levels (Table 3). We found no statistically significant Spearmann's correlation rank coefficient in the group with pneumonia or in the healthy children.

4 Discussion

The aim of this study was to assess the usefulness of copeptin in pediatric patients with

pneumonia. To our knowledge, this is the first report on the sodium-copeptin relationship in children and, to-date, a second one on copeptin in the assessment of pneumonia severity. Copeptin may be helpful in differentiating patients with pneumonia. In a unique study on the subject by Du et al. (2013) median copeptin levels were much higher in children with pneumonia compared with healthy volunteers, and in that area the present study confirmed those data. We consider copeptin to be a potential valuable marker for making clinical diagnosis of pneumonia with sensitivity nearly 90 % and AUC 0.87. Nevertheless, further confirmatory studies are required, in particular research focusing on differences in copeptin levels in patients with pneumonia and other respiratory tract infections, e.g., upper-respiratory tract infection or bronchitis, would make a large contribution to our knowledge on the usefulness of copeptin in CAP.

Copeptin is known to reflect the severity of pneumonia and to predict the mortality in adults (Kolditz et al. 2012; Christ-Crain et al. 2008), and it seem to correspond with complications of pneumonia in children (Du et al. 2013). Our study failed to confirm this observation, as we found no correlation between copeptin and pneumonia severity based on clinical and laboratory features. As there is no widely-used pneumonia severity index in children, we analyzed the clinical and laboratory markers of disease severity used in similar studies and we separately analyzed the risk of complications. It should be emphasized that our investigated group differed from the group of Du et al. (2013); while complications were observed in 21 % of patients (35/165) hospitalized due to pneumonia in that study, in our material only 5 % of children (12 out of the 227) had pleural involvement and most of the patients did not require pleural drainage.

To investigate the sodium-copeptin relationship, we analyzed the whole group and separately the patients with and without pneumonia and with or without hyponatremia. Multivariate logit analysis showed that children with a higher copeptin concentration were at a higher risk of hyponatremia; there was a significant yet weak reverse association ($r = -0.19$). Interestingly, the association was significant only in patients without sodium disorders. Furthermore, there was no association in the CAP group, which suggests that a decrease in sodium is not strictly related to copeptin elevation. A potential explanation may be that in abnormal conditions other mechanisms may cause hyponatremia. Haviv et al. (2005) suggested that the atrial natriuretic peptide (ANP) may play an important role in children with pneumonia by enhancing the diuresis, alongside with natriuresis.

The underlying cause of hyponatremia varies, as lowered sodium concentration may result from excessive water retention or sodium reduction. An increase in the extracellular volume may be caused by excessive fluid intake, free water retention (e.g., in inappropriate antidiuresis) or by a fluid shift from the intracellular compartment. Sodium depletion is caused by sodium losses (e.g., gastrointestinal), insufficient supply, or sodium shift into the intracellular compartment. The syndrome of inappropriate AVP secretion (SIADH) has been shown to be responsible for over 90 % of hyponatremia cases in children with pneumonia (Dhawan et al. 1992). The expected copeptin elevation in hyponatremic patients was confirmed in the current study, although the differentiating causes of hyponatremia could not be reliably settled due to the overlapping values of copeptin concentration. Moreover, Fenske et al. (2009) showed limited usefulness of copeptin in differential diagnosis of the SIADH and sodium-depletion, or sodium and water excess.

In conclusion, there is no linear relationship between the sodium and copeptin levels, although the previously expected copeptin-sodium reverse correlation may be observed, particularly in patients without sodium disorders. Copeptin elevation is not associated with pneumonia severity, at least in mild-to-severe, but rarely complicated cases. Yet, the level of copeptin could be taken into consideration as a possible tool in recognizing pneumonia.

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Etiological Factors Causing Lower Respiratory Tract Infections Isolated from Hospitalized Patients

A. Guzek, Z. Rybicki, K. Korzeniewski, K. Mackiewicz, E. Saks, A. Chciałowski, and E. Zwolińska

Abstract

Lower respiratory tract infections (LRTI) account for 20–30 % of all hospital-acquired contagions. They are characterized by high mortality of hospitalized patients. The most serious form of LRTI is pneumonia, and the most common etiological factors in such cases are bacteria. The article gives the analysis of bacterial flora samples obtained from lower respiratory tract of hospitalized patients. *In vitro* susceptibility of pathogens to selected antibiotics has also been assessed. We carried out a retrospective analysis of 1,171 bacterial strains isolated from 1,171 patients treated in clinics of the Military Institute of Medicine in Warsaw, Poland. In most cases the samples were collected from an endotracheal or tracheostomic tube (71.5 %) and from bronchoalveolar lavage (21.7 %). The most commonly isolated pathogens included *Acinetobacter baumannii* (35.8 %), *Staphylococcus aureus* (27.6 %), *Klebsiella pneumoniae* (19.4 %), and *Pseudomonas aeruginosa* (16.2 %). Multidrug-resistant gram-negative bacteria exhibited 100 % susceptibility to colistin only. *Klebsiella pneumoniae* ESBL+ and *Acinetobacter baumannii* were most susceptible to carbapenems, while *Pseudomonas aeruginosa* strains to ceftazidime. Methicillin-resistant *Staphylococcus aureus* were 100 % susceptible to vancomycin, linezolid, and tigecycline. In conclusion,

A. Guzek, K. Mackiewicz, and E. Saks
Department of Medical Diagnostics, Military Institute of Medicine, Warsaw, Poland

Z. Rybicki
Clinic of Anesthesiology and Intensive Care, Military Institute of Medicine, Warsaw, Poland

K. Korzeniewski (✉)
Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, 4 Grudzińskiego St., Gdynia 81-103, Poland
e-mail: kktropmed@wp.pl

A. Chciałowski
Department of Science, Military Institute of Medicine, Warsaw, Poland

E. Zwolińska
Department of Obstetrics, St Family Maternity Hospital, Warsaw, Poland

identifying the etiological factors causing infections of the lower respiratory tract and determining their drug-susceptibility is of key importance in empirical treatment.

1 Introduction

Lower respiratory tract infections (LRTI) account for 1/3 of all respiratory system contagions. The disease may take an acute form, lasting ≤ 21 days; it is frequently accompanied by cough and at least one of the following pathological symptoms: mucus exertion, shortness of breath, wheezing, or chest pain. The clinical condition of patients suffering from LRTI is serious. The most common etiological factors inducing LRTI are bacterial pathogens; therefore, the majority of infections require the use of antibiotherapy. Severe LRTI infections often coexist with such risk factors as chronic heart disease, lung disease, diabetes, renal failure, cancer, hematologic diseases, or malnutrition (Goulding et al. 2007). The diagnosis and treatment of LRTI requires an interdisciplinary approach which will take into account current clinical, microbiological, and immunological knowledge. Adopting such an approach is necessary due to a large number of etiological factors causing such types of infections, uncharacteristic symptoms in relation to a particular pathogen, a growing number of antibiotic-resistant bacteria, and limited capabilities of microbiological diagnostics. The treatment of respiratory tract infections is at first empirical, an appropriate antibiotic is selected in line with national recommendations taking into account the spectrum of pathogens prevalent in a given area and their resistance patterns (Hryniewicz et al. 2008). The aim of this article is to analyze the bacterial flora sampled from the lower respiratory tract of hospitalized patients. *In vitro* susceptibility of isolated pathogens to selected antibiotics was assessed.

2 Methods

The study was approved by the Ethics Committee of the Military Institute of Medicine in Warsaw, Poland. The retrospective analysis was performed on 1,171 bacterial strains isolated from the lower respiratory tract of 1,171 patients hospitalized in the period July 2010-June 2013 in 20 different clinics of the Military Institute of Medicine in Warsaw, Poland. Samples were collected from an endotracheal or tracheostomic tube (n = 838; 71.5 % of cases), bronchoalveolar lavage (n = 254; 21.7 %), sputum (n = 70; 6.0 %), or pleural fluid (n = 9; 0.8 %). Sputum samples are generally considered as the least reliable, because they often get contaminated with microorganisms inhabiting the oral cavity or throat. Microbiological diagnosis was performed in order to implement appropriate antibacterial treatment. The samples were processed in accordance with generally accepted procedures adopted in microbiological laboratories. We have only analyzed those samples which had been classified as diagnostic (following microscopic examination).

VITEK 2 automated system (bioMerieux, Poland) was used to identify the species of the isolated bacterial strains and to determine their antibiotic susceptibility. The system was used in compliance with the manufacturer's instructions. Drug sensitivity of the strains isolated in 2010 was interpreted in line with the then binding recommendations from the American CLSI (Clinical and Laboratory Standards Institute 2011), while those pathogens which were collected since 2011, were classified according to currently binding European EUCAST guidelines (European Committee on Antimicrobial Susceptibility Testing 2011),

as well as in accordance with the Polish KORLD recommendations (National Reference Center for Antimicrobial Susceptibility 2013). Reference strains: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and ATCC 43300 were used for quality control. The isolates were tested to confirm the presence of the characteristic mechanisms of antibiotic resistance. In case of *Staphylococcus aureus,* resistance to methicillin was confirmed (*methicillin-resistant Staphylococcus aureus*, MRSA), while in case of *Klebsiella pneumoniae* bacilli extended-spectrum beta-lactamases (ESBL) were detected. The diagnostic procedure was in line with the KORLD recommendations.

Statistical elaboration of qualitative variables consisted of chi-squared tests for independence, with Yates' correction for cell counts below 10, and with a check of Cochrane's conditions with Fisher's exact test. Significance of differences was defined as $p \leq 0.05$. A STATISTICA vr. 10.0 commercial package was used for the data comparison.

3   Results

Bacterial strains sampled from 1,171 hospitalized patients contained: *Acinetobacter baumannii* (n = 418; 35.7 %), *Staphylococcus aureus* (n = 322; 27.5 %, including MRSA, n = 108; 9.2 %), *Klebsiella pneumoniae*

(19.6 %, including strains exhibiting ESBL resistance mechanism, n = 126; 10.8 %), *Pseudomonas aeruginosa* (16.1 %), and *Streptococcus pneumoniae* (1.1 %). In more than 70 % of the cases, the etiological factors of lower respiratory tract infections were gram-negative bacteria. Microorganisms isolated from collected samples are presented in Table 1. Bacteria considered as nosocomial pathogens accounted for 98.9 % of the isolates.

In the airway samples, there were significantly more *A. baumannii* strains than *S. aureus* MSSA (p = 0.0001), *S. aureus* MRSA (p = 0.0013), *K. pneumoniae* ESBL− (p = 0.0001), *P. aeruginosa* (p = 0.0001), or *S. pneumoniae* (p = 0.0002). There were significantly more *S. aureus* MSSA strains than *S. aureus* MRSA (p = 0.0011) or *K. pneumoniae* ESBL+ (p = 0.0001), but significantly fewer than *P. aeruginosa* (p = 0.0011). There were significantly fewer *K. pneumoniae* ESBL- strains than *K. pneumoniae* ESBL+ (p = 0.0003) and significantly fewer *K. pneumoniae* ESBL+ strains than *P. aeruginosa* (p = 0.0139). Finally, there were significantly more *K. pneumoniae* ESBL+ strains than *S. pneumoniae* (p = 0.0037). Differences between other isolates obtained from the airways were insignificant.

In the samples obtained from bronchoalveolar lavage, there were significantly fewer *A. baumannii* strains than *S. aureus* MSSA (p = 0.0001) and significantly more *A. baumannii* strains than *S. aureus* MRSA (p = 0.0151), *K. pneumoniae* ESBL−

Table 1 Pathogens isolated from the analyzed biological material (n = 1,171)

Pathogen/material	Specimens collected from artificial airway		Bronchoalveolar lavage		Sputum		Pleural fluid		Total	
	n	%	n	%	n	%	n	%	n	%
A. baumannii	354	42.3	57	22.4	6	8.6	1	11.1	418	35.8
S. aureus MSSA	112	13.4	76	29.9	19	27.1	7	77.8	214	18.3
S. aureus MRSA	77	9.2	25	9.8	6	8.6	0	0.0	108	9.2
K. pneumoniae ESBL−	75	9.0	37	14.6	14	20.0	0	0.0	126	10.8
K. pneumoniae ESBL+	81	9.7	13	5.1	4	5.7	1	11.1	99	8.5
K. pneumoniae KPC	2	0.2	0	0.0	0	0.0	0	0.0	2	0.2
P. aeruginosa	129	15.4	40	15.8	20	28.6	0	0.0	189	16.2
S. pneumoniae	6	0.7	6	2.4	1	1.4	0	0.0	13	1.1
Total	838	100	254	100	70	100	9	100	1,171	100

(p = 0.0001), *P. aeruginosa* (p = 0.0191), or *S. pneumoniae* (p = 0.0011). There were significantly more *S. aureus* MSSA strains than *S. aureus* MRSA (p = 0.0240), *K. pneumoniae* ESBL + (p = 0.0001), or *P. aeruginosa* (p = 0.0015). Also, there were significantly more *K. pneumoniae* ESBL− strains than *K. pneumoniae* ESBL+ (p = 0.0036); significantly more *K. pneumoniae* ESBL+ strains than *S. pneumoniae* (p = 0.0029); and significantly more *P. aeruginosa* strains than *S. pneumoniae* (p = 0.0377). Differences between other isolates obtained from the bronchoalveolar lavage samples were insignificant.

In the sputum samples, there were significantly fewer *A. baumannii* strains than *S. aureus* MSSA (p = 0.0001), *K. pneumoniae* ESBL− (p = 0.0001), or *P. aeruginosa* (p = 0.0001). In the pleural fluid samples, there were significantly fewer *A. baumannii* strains than *S. aureus* MSSA (p = 0.0013) and significantly more *S. aureus* MSSA strains than *K. pneumoniae* ESBL− (p = 0.0402) or *P. aeruginosa* (p = 0.0121). Differences between other isolates were insignificant.

The susceptibility of pathogens to specific antibiotics is presented in Tables 2, 3, and 4. Among non-fermenting bacilli *Pseudomonas*

Table 2 Susceptibility of *Pseudomonas aeruginosa* (n = 189) and *Acinetobacter baumannii* (n = 418) to antibiotics

| | Pseudomonas aeruginosa | | | | Acinetobacter baumannii | | | |
| | Susceptibility | | Resistance | | Susceptibility | | Resistance | |
Antibiotic	n	%	n	%	n	%	n	%
Ciprofloxacin	109	57.7	80	42.3	24	5.7	394	94.3
Imipenem	107	56.6	82	43.4	332	79.4	86	20.6
Meropenem	116	61.4	73	38.6	330	78.9	88	21.1
Ceftazidime	143	75.7	46	24.3	*	*	*	*
Cefepime	109	57.7	80	42.3	*	*	*	*
Piperacillin/tazobactam	45	23.8	144	76.2	*	*	*	*
Colistin	189	100.0	0	0.0	418	100.0	0	0.0
Ampicillin/sulbactam	*	*	*	*	306	73.2	112	26.8
Trimethoprim/sulfamethoxazole	0	0.0	189	100.0	34	8.1	384	91.9

*Not determined due to lack of interpretation according to EUCAST guidelines

Table 3 Susceptibility of *Klebsiella pneumoniae* ESBL− (n = 126), *K. pneumoniae* ESBL+ (n = 101) and *K. pneumoniae* KPC (n = 2) to antibiotics

| | Klebsiella pneumoniae ESBL− | | | | Klebsiella pneumoniae ESBL+ | | | | Klebsiella pneumoniae KPC | | | |
| | Susceptibility | | Resistance | | Susceptibility | | Resistance | | Susceptibility | | Resistance | |
Antibiotic	n	%	n	%	n	%	n	%	n	%	n	%
Ceftazidime	124	98.4	2	1.6	0	0.0	101	100	0	0.0	2	100
Cefotaxime	126	100	0	0.0	0	0.0	101	100	0	0.0	2	100
Imipenem	126	100	0	0.0	99	98.0	2	2.0	0	0.0	2	100
Meropenem	126	100	0	0.0	99	98.0	2	2.0	0	0.0	2	100
SXT	123	97.6	3	2.4	26	25.7	75	74.3	0	0.0	2	100
Piperacillin/tazobactam	115	91.3	11	8.7	4	4.0	97	96.0	0	0.0	2	100
Ciprofloxacin	117	92.9	9	7.1	0	0.0	101	100	0	0.0	2	100
Gentamicin	123	97.6	3	2.4	22	21.8	79	78.2	2	100	0	0.0
Colistin	126	100	0	0.0	101	100	0	0.0	2	100	0	0.0
Tigecycline	119	94.4	7	5.6	67	66.3	34	33.7	2	100	0	0.0

Table 4 Susceptibility of *Staphylococcus aureus* MRSA (n = 108), *S. aureus* MSSA (n = 214) and *Streptococcus pneumoniae* (n = 13) to antibiotics

	Staphylococcus aureus MRSA				*Staphylococcus aureus* MSSA				*Streptococcus pneumoniae*			
	Susceptibility		Resistance		Susceptibility		Resistance		Susceptibility		Resistance	
Antibiotic	n	%	n	%	n	%	n	%	n	%	n	%
Ciprofloxacin	3	2.8	105	97.2	213	99.5	1	0.5	–	–	–	–
Gentamicin	101	93.5	7	6.5	213	99.5	1	0.5	–	–	–	–
Tigecycline	108	100	0	0.0	214	100	0	0.0	–	–	–	–
SXT	108	100	0	0.0	214	100	0	0.0	–	–	–	–
Vancomycin	108	100	0	0.0	214	100	0	0.0	–	–	–	–
Linezolid	108	100	0	0.0	214	100	0	0.0	–	–	–	–
Benzylpenicillin	0	0.0	108	100	59	27.6	155	72.4	13	100	0	0.0
Clindamycin	7	6.5	101	93.5	186	86.9	28	13.1	6	46.0	7	54.0
Erythromycin	–	–	–	–	–	–	–	–	6	46.0	7	54.0
Cefotaxime	–	–	–	–	–	–	–	–	13	100	0	0.0

aeruginosa and *Acinetobacter baumannii* exhibited the highest, 100 % susceptibility to colistin. *Acinetobacter baumannii* was highly susceptible to carbapenems and to sulbactam with ampicillin, whereas *Pseudomonas aeruginosa* demonstrated high susceptibility to ceftazidime (Table 2).

The most effective therapeutic option for the treatment of *Klebsiella pneumoniae* KPC was gentamycin, colistin, or tigecycline, while infections induced by the ESBL− producing bacteria demonstrated high susceptibility to the majority of antibiotics, except for colistin and carbapenems (Table 3). *Staphylococcus aureus* MRSA demonstrated high susceptibility to tigecycline, SXT, vancomycin, and linezolid. The results did not confirm *Pneumococcus pneumoniae* resistance to benzylopenicillin, which is an increasingly common phenomenon in the population of out-patients (Table 4).

4 Discussion

Lower respiratory tract infections (LRTI) account for 20–30 % of all nosocomial infections and cause high mortality in hospitalized patients. The most severe form of the respiratory tract infection is pneumonia. It is diagnosed according to clinical criteria, diagnostic imaging, laboratory and microbiology tests results, which are necessary for the implementation of an appropriate treatment. Each case of pneumonia which occurs 48 h after hospital admission is referred to as hospital acquired pneumonia (HAP) (American Thoracic Society 1996). Ventilator associated pneumonia (VAP) is yet another type of HAP, it occurs 48–72 h after the start of mechanical ventilation. There is also another subtype of HAP, known as health care associated pneumonia (HCAP). It develops in patients hospitalized for at least 2 days in the last 3 months, in persons receiving outpatient intravenous antibiotherapy, or in people living in nursing homes over the last 3 months. The incidence of HAP is 5–15 cases per 1,000 hospitalizations, while the incidence of VAP is 6–20-fold higher due to the presence of airway tubes. Pneumonia occurs in over 25 % of patients treated in intensive care units (ICU), 90 % of whom are intubated. In addition, there has been an increasing number of inpatients diagnosed with community-acquired pneumonia (CAP); those patients are either directly admitted to ICU or are first treated in other hospital wards and then directed to ICU (American Thoracic Society 2005). The development of hospital acquired pneumonia is preceded by the penetration of pathogens into physiologically sterile lower respiratory tract, its colonization, and then overcoming the physiological defense mechanisms. Aspiration of bacterial flora from the oral cavity into the trachea is the first step towards the colonization of the lower respiratory

tract with nosocomial pathogens. The etiology of pneumonia depends on both the saprophytic flora as well as nosocomial pathogens colonizing the oral cavity (American Thoracic Society 1996). Pathogenesis of HAP and VAP depends on a number of different factors, including an earlier use of antibiotics, poor hygiene habits in hospitals, the severity of an infection or the efficiency of the immune system (Rello et al. 2011). HAP is most commonly induced by bacteria. However, viral and fungal infections have also been reported (Celis et al. 1988; Lim and Macfarlane 2001). It has been estimated that gram-negative bacteria are responsible for 50–85 % of all HAP cases, gram-positive bacteria for 20–30 % of the cases, while mixed infections occur in more than 13 % of cases (Intensive Care Antimicrobial Resistance Epidemiology 1996). In early onset pneumonia (EOP), which occurs within the first 4 days after admission to hospital, the most common etiological factors include *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*, methicillin-sensitive *Staphylococcus aureus*, while the late onset pneumonia (LOP), which develops after the fifth day of hospitalization, is usually caused by multidrug resistant *Pseudomonas aeruginosa, Acinetobacter spp.*, *Klebsiella pneumoniae* ESBL (extended-spectrum beta-lactamases), *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* strains (American Thoracic Society 2005). Viral or fungal lung infections occur in patients with impaired immunity, whereas rare cases of anaerobic infections develop in patients in whom aspiration of gastro-intestinal contents occurs.

In a study carried out among patients hospitalized in the Military Institute of Medicine in Warsaw, Poland, multidrug-resistant bacteria predominated, they were mainly bacilli of *Acinetobacter baumannii, Pseudomonas aeruginosa,* as well as *Klebsiella pneumoniae* and *Staphylococcus aureus* (exhibiting antibiotic resistance mechanisms – ESBL, KPC, and MRSA). The specimens were collected from patients who developed pneumonia as a complication of another disease during their hospitalization. This was confirmed by the fact that

98.9 % of the isolates were identified as nosocomial pathogens. The *in vitro* tests indicated that *Klebsiella pneumoniae* ESBL strains can be successfully treated with carbapenems. This has also been confirmed by other studies which show that carbapenems are an effective method of treating HAP across Europe (Hryniewicz and Ozorowski 2011). In the present study, *Non-Enterobacteriaceae* bacilli exhibited 100 % susceptibility to colistin; a drug which is usually used as the last resort against infections caused by gram-negative bacteria that are resistant to other antibiotics. Due to its poor tissue penetration, it is recommended that colistin be administered simultaneously both intravenously and in aerosol. Additional observations indicate that nephrotoxicity associated with the use of colistin is not as common as was previously thought (Rybicki 2012). *Acinetobacter baumannii* sensitivity to sulbactam is also worth mentioning. Despite its *in vitro* susceptibility, clinical efficacy of the drug is controversial because of the rapidly increasing resistance. Methicillin-resistant *Staphylococcus aureus* were 100 % susceptible to vancomycin, linezolid, and tigecycline. The American Thoracic Society (2005) recommend the use of both vancomycin and linezolid in the treatment of hospital acquired pneumonia caused by *Staphylococcus aureus* MRSA, provided that the concentration of vancomycin throughout the therapy is a minimum of 15–20 μg/ml (Liu et al. 2011). Given the increasing vancomycin MIC values, this parameter should be obligatorily marked in all severe cases caused by staphylococci MRSA. Several studies have concluded that the mortality in some severe cases may be decreased by 16–22 %, if linezolid is used instead of vancomycin. This may be due to the ability to achieve a 3.5-fold higher concentration of linezolid in lungs in proportion to its concentration in the blood serum, as opposed to vancomycin, whose concentration in the lungs is lower than in the serum. With regard to tigecycline, it should be remembered that this drug has not been approved for the treatment of lung infections. However, there have been reports on the use of tigecycline in the treatment of VAP in patients for whom

there were no other therapeutic options (Kelesidis et al. 2008). A small number of *Streptococcus pneumoniae* strains isolated in the present study was due to the fact that the majority of culture samples came from patients with pneumonia which occurred as a secondary complication of other health problems, mainly surgical or traumatic, and was induced by nosocomial bacteria. The majority of samples we studied were collected from an endotracheal or tracheostomic tube (71.5 %). This method, according to many authors, is comparable to bronchoalveolar lavage (21.7 %); a technique regarded as the gold standard (Canadian Critical Care Trials Group 2006; Kowalczyk et al. 2011). In the majority of cases we found that the etiological agent responsible for the development of lower respiratory tract infections were gram-negative bacteria, which is consistent with the results obtained in most hospitals around the world (Rybicki 2012). Our findings regarding antibiotic resistance are also in line with test results reported by other authors (Duszyńska 2010; Płusa 2013). The study helps implement more effective empirical treatment of respiratory infections in hospitalized patients.

5   Conclusions

The most common pathogens which were isolated from the lower respiratory tract of hospitalized patients included gram-negative bacteria: *Acinetobacter baumannii, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Multidrug-resistant gram-negative bacteria exhibited 100 % susceptibility to colistin only. Acquiring accurate data on drug-susceptibility of pathogens and their resistance patterns allows optimizing the empirical hospital treatment of respiratory infections pending the results of microbiological tests.

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Antibiotic Consumption Pattern in the Neonatal Special Care Unit Before and After Implementation of the Hospital's Antibiotic Policy

A. Nitsch-Osuch, D. Kurpas, E. Kuchar, K. Życińska, T. Zielonka, and K. Wardyn

Abstract

Current and detailed knowledge of antibiotic use is essential in order to implement strategies for reducing the overuse of antibiotics. The objective of our study was to determine the effectiveness of the implementation of the hospital antibiotic policy (HAP) by assessing antibiotic consumption in the Special Neonatal Care Unit (SNCU) in Warsaw, Poland, before and after this intervention. Antibiotic use was calculated in daily defined doses (DDDs) per 100 patient-days and DDDs per 100 admissions. The antibiotics were ranked by volume of DDDs and the number of antibiotics, which accounted for 90 % and 100 % of the total volume, respectively: DU90% and DU100% (where DU stands for drug use). Total antibiotic consumption increased slightly after the introduction of the HAP: the total DDDS was 707.87 and 753.12 in 2011 and 2012, while the number of DDDs/100 admissions was 352.17 and 369.12 in 2011 and 2012, respectively. After the introduction of the HAP, an increase in ampicillin and aminoglycoside use was observed, along with a reduction in the DU100% and DU90% rates (15 *vs*. 9 and 4 *vs*. 3, respectively). The introduction of the HAP resulted in changes in antibiotic consumption patterns, but the general antibiotic consumption density remained the same.

A. Nitsch-Osuch (✉), K. Życińska, T. Zielonka, and K. Wardyn
Department of Family Medicine, Warsaw Medical University, 1A Banacha St., Block F, 02-097 Warsaw, Poland
e-mail: anitsch@amwaw.edu.pl

D. Kurpas
Department of Family Medicine, Wroclaw Medical University, Wroclaw, Poland

Public Higher Medical Professional School, Opole, Poland

E. Kuchar
Department of Paediatric Infectious Diseases, Wroclaw Medical University, Wroclaw, Poland

1 Introduction

Neonatal infection is a significant cause of morbidity and mortality and results in 1.6 million neonatal deaths per year worldwide (Vergano et al. 2005). Antibiotics are the most commonly used medicines in neonatal intensive care units (NICUs) and Special Neonatal Care Units (SNCUs) (Warrier et al. 2006). High antibiotic exposure rates (75–95 %) have been reported, which are most probably based on the common practice of administering antibiotics to sick neonates, pending bacterial culture results, and to neonates with risk factors for developing infectious diseases. However, antibiotic resistance is becoming one of the major problems during the use of antibiotics (Morens and Fauci 2012). It has previously been shown that inappropriate use of antibiotics is the predominant factor in increased antimicrobial resistance; therefore, it is important to prevent or minimize the occurrence of antimicrobial-resistant bacteria. It has also been reported that inappropriate use of antibiotics in hospitals ranges from 26 to 57 % (Niwa et al. 2012; Arnold 2005). However, antimicrobial stewardship programs are known to promote appropriate use of antibiotics and two core proactive evidence-based strategies are recommended by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA), which are essential for hospitals: formulary restriction and preauthorization, and auditing with intervention and feedback (Niwa et al. 2012).

Current and detailed knowledge of antibiotic use is necessary to implement strategies for reducing the overuse and misuse of antibiotics as well as the spreading of resistant microorganisms. Antibiotic consumption analysis is fundamental for the assessment of therapy costs, creation of drug policies, and for analysis of bacterial resistance in hospital environments (Kuster et al. 2008; Cars et al. 2001).

There have been very few studies describing antibiotic use in NICUs and SNCUs, especially in Poland and other Eastern and Central European countries. An antimicrobial stewardship programs have still not been implemented in a number of medical institutions despite being highly recommended by the Polish Ministry of Health and the Sanitary Chief Inspectorate (Hryniewicz and Ozorowski 2011).

The objective of our study was to determine the effectiveness of the hospital antibiotic policy (HAP) by assessing the antibiotic consumption in the Special Neonatal Care Unit (SNCU) in Warsaw, Poland over a 2-year period, before and after its introduction.

2 Methods

Approval from a local Ethics Committee was obtained prior to the study. A retrospective analysis of antibiotic consumption at the SCNU before and after the introduction of the HAP was conducted. The period analyzed was 2011 (a year before the hospital antibiotic policy was introduced) and 2012 (the first year after the introduction of the HAP). The SNCU is a 10-bed second level unit providing care for newborns born at the hospital and referred from the first level units (for diagnostic and therapeutic procedures) and third level units (for continuation of treatment and rehabilitation). The average number of live births at the hospital is 4,000 per year and approximately 5 % of newborns are admitted to the SNCU. A total of 418 newborns that required antibiotic therapy were hospitalized in the SNCU (208 in 2011 and 210 in 2012, respectively). The patients were treated with antibiotics due mainly to prematurity and infections (Table 1).

In 2012, an extensive antibiotic policy program created by the members of the hospital's infection control team (ICT) was implemented. The ICT members included a physician (an epidemiology and pediatrics specialist), a clinical pharmacist, a clinical microbiologist, and an epidemiological nurse. Before introduction of the antibiotic policy, the hospital informed the Drugs and Therapeutics Committee (DTC) and a written antibiotic formulary defined as a list of antibiotics used in a hospital was established. The HAP was defined as written

Table 1 Diagnoses of newborns hospitalized in the SCNU treated with antibiotics[a]

| | Year | | |
| | 2011 | 2012 | |
Diagnoses	Number of patients receiving the diagnosis		2011–2012
Pneumonia	15 (4 %)	10 (3 %)	25 (3 %)
Prematurity and intrauterine infection	194 (53 %)	187 (51 %)	381 (52 %)
Infant respiratory distress syndrome	78 (21 %)	90 (25 %)	168 (23 %)
Meconium aspiration syndrome (MAS)	2 (0.5 %)	2 (5 %)	4 (0.5 %)
Intrauterine infection[b]	73 (20 %)	72 (20 %)	145 (20 %)
Sepsis	1 (0.3 %)	3 (8 %)	4 (5 %)
Total	363 (100 %)	364 (100 %)	727 (100 %)

[a]The number of diagnoses does not equal the number of newborns requiring antibiotic therapy because of the possibility of multi-diagnoses in a single patient

[b]Diagnosed in mature newborns

guidelines for prescribing antibiotics. The antibiotics used in the hospital were divided into three groups: first line (1st line) antibiotics (e.g., penicillins, 1st generation cephalosporins), second line (2nd line) antibiotics (e.g., macrolides, 2nd and 3rd generation cephalosporins, amoxicillin with clavulanic acid, piperacillin with tazobactam), and third line (3rd line), restricted antibiotics only available after pre-authorization (or additional authorization) by a physician from the infection control team (e.g., glicopeptides, carbapenems).

Data on the quantitative and qualitative use of antibiotics in 2011 and 2012 were reported by the hospital's pharmacy. The data represented the dispensing of antibiotics from the hospital pharmacy to the Special Neonatal Care Unit. The annual report on the total volume of antibiotics prescribed was analyzed. The antibiotic use was calculated in daily defined doses (DDDs) per 100 patient-days and DDDs per 100 admissions according to the Anatomical Therapeutic Chemical Classification (ATC) System, from the WHO, version 2009. Furthermore, antibiotics were ranked by volume of DDDs and the number of antibiotics, which accounted for 90 % and 100 % of the total volume (DU90% and DU100%, respectively, where DU stands for drug use). The non-parametric variables were analyzed using the χ^2 test with a p-value of <0.05 considered as statistically significant. The data were analyzed using an SPSS commercial package (SPSS Inc., Chicago, IL).

3 Results

Total antibiotic consumption at the SCNU increased slightly after the introduction of the Antibiotic Policy. The total DDSs were 707.87 in 2011 and 753.12 in 2012. The number of DDDs/100 admissions was 352.17 in 2011 and 369.12 in 2012 (Table 2).

After the introduction of the HAP in 2012 (*vs.* 2011), a significant (p < 0.05) increase in the use of ampicillin and aminoglycosides (mainly gentamicin) was observed with a simultaneous decrease in the use of penicillins with beta-lactamase inhibitors (amoxicillin with clavulanic acid). The use of macrolides and meropenem was also reduced after the implementation of the antibiotic stewardship. Both DU100% and DU90% were reduced (15 *vs.* 9 and 4 *vs.* 3, respectively) (Table 3).

4 Discussion

To address the growing problem of antibiotic overuse and resistance, the Center for Disease Control and Prevention, the National Foundation for Infectious Diseases, the Infectious Diseases Society of America, and the Polish Ministry of Health have urged all hospitals to develop systems to monitor antibiotic use and have encouraged physicians to reduce the inappropriate use (Hryniewicz and Ozorowski 2011; Arnold 2005). Our study is the first research

Table 2 Antibiotic consumption pattern before and after implementation of the hospital antibiotic policy (HAP)

Antibiotics for systemic use	DDDs		DDDs/100 patient days		DDDs/100 admissions		% of DDDs		
Year	2011	2012	2011	2012	2011	2012	2011	2012	p
Penicillins:	277.1	470	11.3	62.4	137.8	230.3	39 %	62.4 %	0.001
Ampicillin	275.1	470	11.3	62.4	136.9	230.3	38.8 %	62.4 %	0.001
Piperacillin	0.21	0	0.01	0	0.1	0	0.03 %	0 %	>0.05
Cloxacillin	1.75	0	0.07	0	0.9	0	0.2 %	0 %	>0.05
Penicillins with beta-lactamase inhibitors:	294.8	62.4	12.1	8.2	146.7	3.3	41.6 %	7.9 %	0.001
Amoxicillin with clavulanic acid	288	62	11.8	8.2	143.3	3.3	40.7 %	8.2 %	0.001
Piperacillin with tazobactam	6.84	9.42	0.3	0.03	3.4	0.03	1.0 %	0.3 %	>0.05
Aminoglycoside:	85.1	188.1	3.5	24.9	42.3	14.2	12 %	24.9 %	0.018
Amikacin	45	4.75	1.8	0.6	22.4	2.3	6.4 %	0.6 %	>0.05
Gentamicin	26.6	183.3	1.1	24.3	13.2	11.9	3.8 %	24.3 %	0.001
Netilmycin	13.5	0	0.6	0	6.7	0	1.9 %	0 %	>0.05
2nd generation cephalosporin: Cefuroxime	4	4.8	0.2	0.9	1.9	0.9	0.6 %	0.2 %	>0.05
3rd generation cephalosporins:	15.1	22.1	0.6	2.7	7.5	1.4	2.1 %	2.9 %	>0.05
Cefotaxime	0.8	0	0.03	0	0.4	0	0.1 %	0 %	>0.05
Ceftazidime	14.4	22.1	0.6	2.7	7.2	1.4	2 %	2.9 %	>0.05
Macrolides:	18	0	0.7	0	8.9	0	2.5 %	0 %	>0.05
Erythromycin	3	0	0.1	0	1.5	0	0.4 %	0 %	>0.05
Clarithromycin	15	0	0.6	0	7.5	0	2.1 %	0 %	>0.05
Glycopeptide – vancomycin	8.8	6.3	0.4	0.8	4.3	0.4	1.2 %	0.8 %	>0.05
Carbapenem – meropenem	5	0.3	0.2	0.3	2.5	0.2	0.7 %	0.3 %	>0.05
Total	707.9	753.1	28.9	30.8	352.2	369.1	100 %	100 %	–

DDD daily defined doses

describing the effect of the introduction of the hospital antibiotic policy on the antibiotic use among patients of the SNCU in Poland and other Eastern European countries. It has been previously described that policies and practices relating to antibiotic stewardship vary considerably across European hospitals. Hospitals in northern and western Europe were more likely to appoint antibiotic committees and policies than those from southern and south-eastern Europe. It was found that more work is required and recommended to achieve harmonization of recommended practices, particular in eastern European hospitals (Bruce et al. 2009).

One year after the introduction of the antibiotic policy, total antibiotic consumption at the SCNU did not decrease, but it increased slightly (707.865 and 753.12 DDDs in 2011 and 2012, respectively). Our results may be surprising;

however, other authors have reported similar findings. An increase of 25 % in the antibiotic use after the introduction of similar interventions has been reported (Gyssens et al. 1997). Manuel et al. (2010) showed that antimicrobial intervention is associated with a shorter duration of antibiotic treatment regardless of changes in the density of antimicrobial use. Niwa et al. (2012) also failed to observe a decrease in the density of antibiotic use in the first year after the introduction of the antibiotic policy. A possible explanation for this result may be that the observation period was too short.

In the present study, the antibiotic use expressed by DDDs/100 admissions was similarly high before and after the introduction of the HAP (352.17 DDDs/100 admissions in 2011 and 369.12/100 admissions in 2012). Liem et al. (2010) have reported lower or similar use

Table 3 Drug use rates before and after the introduction of the hospital antibiotic policy (HAP)

Year	DU100%	DU90%
2011	**15:**	**4:**
	Ampicillin	Ampicillin
	Piperacillin	Amoxicillin with clavulanic acid
	Cloxacillin	Amikacin
	Amoxicillin with clavulanic acid	Gentamicin
	Piperacillin with tazobactam	
	Amikacin	
	Gentamicin	
	Netilmicin	
	Cefuroxime	
	Cefotaxime	
	Ceftazidime	
	Erythromycin	
	Clarithromycin	
	Vancomycin	
	Meropenem	
2012	**9:**	**3:**
	Ampicillin	Ampicillin
	Gentamicin	Gentamicin
	Amoxicillin with clavulanic acid	Ampicillin with clavulanic acid
	Piperacillin with tazobactam	
	Amikacin	
	Cefuroxime	
	Ceftazidime	
	Vancomycin	
	Meropenem	

DU drug use

of antibiotics in neonatal wards (from 63.0 to 230.7 DDDs/100 admissions). After the introduction of the HAP we did not observe a reduction in the antibiotic use measured by the number of DDDs per 100 admissions and DDDs per 100 hospitalizations. However, we reported a decrease in the number of antibiotic in the DU100% and DU90% segments and changes in antibiotic consumption patterns. We assume that the reduction in DU100% and DU90% rates could be a positive result of the HAP introduction.

It should be pointed out that the number of antibiotics in the DU100% and DU90% segments were relatively low (DU100% 15 in 2011 and DU100% 9 in 2012; DU90% 5 in 2011 and 4 in 2012). Dutch researchers reported DU90% from 3 to 10 (Liem et al. 2010), while Chinese researchers indicated higher rates (varying between 16 and 20) (Zhang et al. 2008). Russian researchers reported DU90% 8 and DU100% 22 and data from Croatia indicated DU90% 11 and DU100% 35 (Palcevski et al. 2004). Particularly, DU90% is considered an important tool for assessing the quality of drug prescription. In addition to the numbers of drugs in the DU90% segment, the presence of, and adherence to, treatment guidelines may serve as the general quality indicators (Bergman et al. 1998).

The most commonly used antibiotics at the NICU in the present study were ampicillin followed by aminoglycosides. These results are in agreement with those obtained by others: Liem et al. (2010) found that penicillin (mainly ampicillin, amoxicillin, and amoxicillin with

clavulanic acid) and aminoglycosides (mainly gentamicin and amikacin) were the most commonly used antibiotics in Dutch NICUs. Our results are also similar to those described by other Polish researchers and indicate that betalactams and aminoglycosides are the most commonly used groups of antibiotics among neonates (Różańska et al. 2012). The result is not surprising since these antibiotics are included in the guidelines for treatment of neonatal sepsis, meningitis, and necrotizing enterocolitis; the main indications for antibiotic therapy among newborns (Gordon and Isaacs 2004; Stoll et al. 2002). A low use of vancomycin and meropenem should be highlighted in our study as a positive outcome. Vancomycin should be reserved for episodes including microorganisms confirmed as resistant to penicillin (Fernando et al. 2008). In the NICUs much of the overuse involves vancomycin, as the frequency of coagulase-negative staphylococci infections make it a popular choice for empirical therapy. Vancomycin was used in a very rational way in our study – only during microbiologically evident infections caused by resistant staphylococci. The use of vancomycin ranged from 0.82 to 1.23 % in our patients and this rate was similar to that reported by Rożańska et al. (2012). We also reported a low use of cefotaxime, which should also be considered as a positive observation. Reports from the UK indicate that 100 % of isolates responsible for early onset sepsis were susceptible to amoxicillin and cefotaxime, and 94 % were susceptible to penicillin and gentamicin. However, despite an overall susceptibility to a combination of amoxicillin and cefotaxime, cefotaxime should not be used in the empirical regimen because of its ability to promote bacterial resistance (Clark et al. 2006). Ampicillin and gentamicin should be used as the most common antibiotics in neonates (Tripathi et al. 2012).

The limitations of this study should be mentioned. We measured the antibiotic use in the SNCU in adult Defined Daily Dose. The DDD as assigned by the World Health Organization has been the most commonly used unit for quantifying measurements in various settings, and it has shown its value for this purpose. The DDD is the assumed average maintenance dose per day for a drug in its main indication for adults and it is commonly expressed with a certain population size denominator such as patient days, bed days, admission days, or inhabitant days (Liem et al. 2010). However, the main disadvantage is that the DDD reflects neither the recommended or actually prescribed daily dose (PDD) for individual patients nor a specific patient population. The second shortcoming of the DDD methodology is its applicability in pediatrics and neonatology since the dosing of antibiotics is based on body mass. The WHO's International Working Group for Drug Statistics Methodology released the opinion that it is not possible to define pediatric DDDs because dose recommendations for use in children vary according to age and body mass (Liem et al. 2010). In response to the WHO's negative comments about pediatric DDDs, several alternative measurement systems for the antibiotic use in children have been proposed, such as an estimation of antibiotic exposure by controlling the patient's weight and amount of waste drug. However, some authors proposed a calculation of neonatal DDDs for commonly used top 10 antibiotics in neonates based on an assumed neonatal weight of 2 kg. Some researchers have already used the adult DDDs in order to describe antibiotic consumption in the NICU, since the range of body weight in the neonatal population does not vary significantly and antibiotic doses do not fluctuate (Liem et al. 2010).

Another limitation is calculations based on purchase data from the hospital pharmacy. We lacked data on the antibiotic use at an individual patient level. However, we hope that with the increasing use of computerized medical information systems it will be considerably easier to get access to data on an individual therapy level, such as days of therapy. Similar limitations were also reported in other studies (Liem et al. 2010). We should also underline that our study period was only 2 years, which may be too short to indicate significant changes in the general density of antibiotic consumption. Our results indicate a relatively high constant consumption of antibiotics in the SNCU and may serve as a background for future comparisons describing the consumption of antibiotics in the

same ward at different periods and for comparisons with other hospital wards in Poland and other countries.

5   Conclusions

The consumption of antibiotics in the Special Neonatal Care Unit remains relatively high despite the introduction of the Hospital Antibiotic Policy. This policy is effective in spurring desirable changes of antibiotic consumption patterns. Antibiotic stewardship should be implemented, but it needs a constant feedback and improvement to be effective.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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Respiratory Tract Infections in Children in Primary Healthcare in Poland

E. Kuchar, K. Miśkiewicz, L. Szenborn, and D. Kurpas

Abstract

Respiratory tract infections are the most common diseases in children. The aim of the study was to assess their frequency and antibiotic treatment in Poland. We retrospectively analyzed 91 randomly-selected children aged 0–17 years receiving care from birth in a large primary healthcare establishment in the city of Wroclaw in Poland. Respiratory tract infections were responsible for 25–40 % of all primary healthcare visits. The median of visits due to upper respiratory tract infections was 1.8 per year in all children and 2.0 per year in children 0–3 years old. Antibiotics were overused; the majority (57.4 %) of the respiratory infections were treated with antibiotics: acute tonsillitis in 90.7 %, bronchitis in 67.5 %, otitis media in 65.9 %, pneumonia in 60.9 %, non-specific upper respiratory tract infections in 25.8 %, laryngitis in 22.2 %, and sinusitis in 12.5 %. The higher the number of antibiotic therapies, the higher the total number of visits including visits due to respiratory tract infections. In conclusion, implementation of careful and responsible management of a rational use of antibiotics is urgently needed since a reduction in their use may lead to a decrease in the number of visits due to upper respiratory tract infections and a total number of primary care visits.

1 Introduction

Respiratory infections: common cold, acute tonsillitis, acute rhinosinusitis, acute otitis media, flu-like illness, acute bronchitis, and pneumonia are the most common diseases in human beings including infants and children (Heikkinen and Järvinen 2003). Despite their general benign nature, they are a major cause of morbidity and mortality worldwide, and a significant burden on

E. Kuchar, K. Miśkiewicz (✉), and L. Szenborn
Department of Pediatric Infectious Diseases, Medical University of Wroclaw, 44 Bujwida St., Wroclaw, Poland
e-mail: kj.miskiewicz@gmail.com

D. Kurpas
Department of Family Medicine, Medical University of Wroclaw, Wroclaw, Poland

Public Higher Medical Professional School in Opole, Opole, Poland

society (Wat 2004). Colds or non-specific upper respiratory tract infections (URTI) stand out from the above outlined ailments quite strikingly. The common cold is an acute, self-limiting viral infection of the upper respiratory tract. Typical symptoms include sneezing, nasal congestion and discharge, sore throat, cough, low grade fever, headache, and malaise (Heikkinen and Järvinen 2003). Rhinoviruses with over 100 serotypes and accounting for 30–50 % of common colds are the most common etiologic agents. Coronaviruses are responsible for 10–15 % and influenza viruses for 5–15 % of upper respiratory tract infections (URTI) (Zambon et al. 2001). While adults suffer from two to five common colds per year, infants and schoolchildren suffer from seven to ten common colds annually (Johnston and Holgate 1996). Although there are many possible complications, including sinusitis and middle ear involvement, nasal bleeding, and exacerbation of underlying chronic conditions like asthma, cystic fibrosis or chronic bronchitis, the infection is generally self-limiting and lasts up to 10 days (Fashner et al. 2012). Acute pharyngitis, a so-called sore throat, is a common element of respiratory infections. It is often the first symptom of URTI. When bacterial streptococcal etiology is suspected, acute tonsillitis is usually diagnosed. Acute rhinosinusitis is a symptomatic inflammation of the mucosa of the paranasal sinuses and nasal cavity. Uncomplicated rhinosinusitis, without any clinically evident extension of the inflammation outside the paranasal sinuses and nasal cavity, usually accompanies common colds (Wald et al. 2013). Acute otitis media is a viral or bacterial infection of the middle ear. Uncomplicated acute otitis media may accompany common colds. Many respiratory infections show a seasonal variation in incidence, for example influenza and respiratory syncytial virus (RSV) epidemics occur predominantly in the winter months, with a January–March peak (Wat 2004).

In Poland, primary healthcare for infants and children (below the age of 18 years) is provided by pediatricians or family doctors. In large cities, access to primary care is easy, free of charge, and children are usually cared for by a pediatrician.

Respiratory infections are usually diagnosed clinically, based on symptoms. Although the symptoms of respiratory infection are well known by patients and their parents, and self-diagnosis with self-treatment is common, these infections are responsible for a significant number of primary healthcare visits. URTIs are treated mainly symptomatically, but use of antibiotics is quite common; a number of studies have documented a widespread use of antibiotics in outpatient clinics for putative viral URTIs (Mainous et al. 2003; Gonzales et al. 2001; McCaig and Hughes 1995). There are some indications that Polish primary care physicians also put overmuch desire into prescribing antimicrobials in respiratory infections (Windak et al. 1996; Touw-Otten and Johansen 1992).

Information about the burden of respiratory infections and their treatment with antimicrobials is essential because of logistical issues (organization and costs of healthcare for children) and preservation of antibiotics. It has also been shown that a decrease in the antibiotic prescription rate might lead to a decline in respiratory infections presented in general practice (Fleming et al. 2003). Therefore, the aim of the present study was to assess the burden of respiratory infections in children in primary care in Poland focusing on the antibiotic overuse.

2 Methods

The study protocol was approved by a local Ethics Committee and the personal data of all children were protected. We conducted a sample survey in a large primary healthcare establishment caring for about 2,000 children in the city of Wroclaw, Poland. We retrospectively analyzed the medical charts of 91 randomly-selected children (including 48 girls, 52.7 %) aged 0.7–17.1 years (3.9 ± 2.8 years, median 4.2, IQR 3.93). The age distribution in the study group is presented in Table 1.

The selected primary healthcare establishment was staffed by three full-time pediatricians and characterized by a high staff turnover, i.e., many different physicians worked in the establishment

Table 1 Age distribution in the study group

	Girls (n = 48)		Boys (n = 43)	
Age	n (%)		n (%)	
≤Q25 %	10	20.8	13	30.2
≤Q50 %	15	31.2	9	20.9
≤Q75 %	10	20.8	11	25.6
>Q75 %	13	27.1	10	23.3

Q quartile

Wilcoxon rank-sum test W = 1,117.5, p = 0.494; Fisher exact test, p = 0.574

Table 2 Frequency of antibiotic therapy

	Therapy			
	Antibiotics		No antibiotics	
Diagnosis	n (%)		n (%)	
Non-specific URTI	206	25.8	594	74.2
Acute tonsillitis	39	90.7	4	9.3
Otitis media	56	65.9	29	34.1
Bronchitis	79	67.5	38	32.5
Sinusitis	1	12.5	7	87.5
Laryngitis	4	22.2	14	77.8
Pneumonia	14	60.9	9	39.1

URTI upper respiratory tract infections

during the period analyzed. Then we drew two letters from the alphabet and selected children with a family name beginning with those letters. Only children cared for from birth were included. Most patients were born in 2008 (20/91, 21.9 %). We analyzed the number of visits due to respiratory tract infections, the mean age at which the first infection occurred, and the type of infections. The number of infections treated with antibiotics was also assessed.

Categorical variables were reported as frequencies (%). Qualitative data were not normally distributed, which was confirmed by the Shapiro-Wilk test, and were presented as median, minimum–maximum (min–max), and interquartile range (IQR). Differences in the qualitative variables between groups was verified by the Wilcoxon rank-sum test or the Kruskall-Wallis rank-sum test. Independence of categorical variables was confirmed by the Fisher exact test. The Spearman rank correlation test was used to check correlations between the variables. The level of statistical significance was set at $p < 0.05$. The R 2.10.1 (for Mac OS X Cocoa GUI) statistical software was used for all data analyses.

3   Results

The median number of all visits was 32 (min–max 5–136, IQR 30). The median of the annual indicator of total visits (number of visits divided by child's age at the study end) was 8.3 per year (min–max 2.1–29.8, IQR 5.5). The median of all visits due to respiratory tract infections was 13 (min–max 1–77, IQR 15.5), with the median of the annual indicator being 2.6 per year (min–max 0.15–11.9, IQR 3.4). The median of visits due to URTI was 8 (min–max 1–33, IQR 9.5), with the median of the annual indicator being 1.8 per year (min–max 0.2–6.0, IQR 1.8).

The distribution of visits due to URTIs in the first 3 years of life was similar and not related to gender (W = 919, p = 0.371). The median of visits due to URTIs was 2.0 per year (min–max 0.0–9.0, IQR 2.0) in the first year of life, 2.0 per year (min–max 0.0–8.0, IQR 3.0) in the second year of life and 2.0 per year (min–max 0.0–11.0, IQR 4.0) in the third year of life. The medians did not differ significantly (Kruskal-Wallis chi^2 = 1.4456, df = 2, p = 0.485).

The median of the age at which the first respiratory infection occurred was 0.4 years (min–max 0.0–4.6, IQR 0.6), while the median of the first antibiotic treatment was 0.7 years (min–max 0.1–4.6, IQR 0.7). The vast majority of children, 84.6 % (77/91), were treated with antibiotics and the first antibiotic was prescribed due to a respiratory tract infection in most of them 60/91 (65.9 %).

The majority (57.4 %) of respiratory tract infection episodes were treated with antibiotics. The most common non-specific URTIs were treated with antibiotics in 25.8 % of cases. Antibiotics were prescribed in 90.7 % cases of acute tonsillitis, 67.5 % of bronchitis, 65.9 % of otitis media, 60.9 % of pneumonia, 22.2 % of laryngitis, and 12.5 % of sinusitis (Table 2). The annual antibiotic therapy indicators (number of antibiotic therapies due to a given diagnosis divided by child's age at the study end) was highest in acute tonsillitis (median 0.38) as presented in Table 3.

Table 3 Annual indicators of therapy with antibiotics

Annual indicators of antibiotic therapy[a]	Median	Min–max	IQR
Acute tonsillitis	0.38	0.00–1.73	0.61
Otitis media	0.00	0.00–0.72	0.03
Bronchitis	0.00	0.00–1.00	0.17
Sinusitis	0.00	0.00–1.24	0.20
Laryngitis	0.00	0.00–0.51	0.00
Pneumonia	0.00	0.00–0.69	0.00

[a]Number of antibiotic therapies due to a given diagnosis divided by the age of a child at the end of the study

The median of the annual antibiotic therapy indicators (number of antibiotic therapies divided by child's age at the study end) was 0.8 per year (min–max 0.0–3.9, IQR 1.2). The median of the annual antibiotic therapy indicators due to URTI (number of antibiotic therapies due to URTI divided by child's age at the study end) was 0.6 per year (min–max 0.0–3.1, IQR 1.0). Acute tonsillitis was the respiratory infection most commonly treated with antibiotics (Kruskal-Wallis $chi^2 = 170.3527$, $df = 6$, $p < 0.0001$). Children treated with antibiotics due to URTI had significantly higher annual indicators for visits due to URTI ($W = 720.5$, $p = 0.046$).

3.1 Significant Correlations

The annual indicator of the total number of visits correlated with the number of visits due to URTIs ($r = 0.71$, $p < 0.0001$) – the higher the number of visits due to URTI, the higher was the number of visits to the primary care establishment. Interestingly, the annual indicator of the total number of antibiotic therapies was associated with the annual indicator of the total number of visits ($r = 0.57$, $p < 0.0001$) – more often use of antibiotics was reflected in more visits to the doctor. We also calculated that the annual antibiotic therapy indicator due to URTI correlated with the annual indictor for the total number of visits ($r = 0.52$, $p < 0.0001$) – more often antibiotic treatment was prescribed due to URTI, the more often visits were made to the doctor.

Administration of the first antibiotic in life due to a respiratory tract infection had no impact on the annual indicator for visits due to URTI ($W = 386$, $p = 0.644$).

We also observed a significant correlation between the annual indicator for the total number of antibiotic therapies and the annual indicator for visits due to URTI ($r = 0.64$, $p < 0.0001$) – the higher the number of antibiotic therapies, irrespective of the reason, the higher was the number of visits due to URTI. Similarly, the annual indicator of antibiotic therapies due to URTI correlated with the annual indicator of visits due to URTI ($r = 0.60$, $p < 0.0001$) – the higher the number of antibiotic therapies due to URTI, the higher was the number of visits due to URTI.

The age of the first respiratory infection visit correlated with the annual indicator for visits due to URTI ($r = -0.39$, $p < 0.001$) – the older the patient at the time of the first respiratory infection the less often visits were made due to URTI. Similarly, the age at which the first antibiotic treatment was prescribed correlated with the annual indicator for visits due to URTI ($r = -0.27$, $p = 0.018$) – the older the patient at the time of the first treatment with antibiotics, irrespective of the reason, the less often visits were made to URTI. On the other hand, whether or not the URTI was the reason for the first ever visit to a primary healthcare establishment had no impact on the number of visits due to URTI ($W = 314.5$, $p = 0.085$).

4 Discussion

There is relatively little information on the burden of respiratory tract infections in children in primary care and their treatment with antibiotics, which may be overused (Kardas et al. 2005; Windak et al. 1996). Fragmentary and incomplete information on the epidemiology of URTIs in children in Poland comes from single studies (Tranda et al. 2000). Polish recommendations regarding the treatment of respiratory tract infections are based mainly on international data (Commission on Respiratory Tract Diseases,

Committee on Clinical Pathophysiology of the Polish Academy of Sciences 2008; Hryniewicz et al. 2010). That is why our study, although small and performed in a single center, adds valuable information on this subject. We estimate that URTIs are responsible for about two visits per year to primary care establishments in Polish children (median 1.8 per year in all age groups, 2.0 per year in the first 3 years of life). It is less than reported in the literature. According to the most commonly cited authors, infants and school children suffer from seven to ten common colds annually (Johnston and Holgate 1996). The explanation for this difference is self-treatment of generally benign URTIs, e.g., common colds without consulting a primary care physician. Since the symptoms of respiratory infection (sneezing, cough, or hoarseness) are well known by patients and their parents, self-diagnosis, followed by self-treatment, is common. Thus, the number of primary healthcare visits depends not only on the number of URTIs, but also on the threshold for consultation with a pediatrician, i.e., the willingness to consult the primary care physician and the ease of access to a healthcare establishment. Our results are more consistent with the results obtained in preschool children in Norway. In a study by Kvaerner et al. (2000), URTIs were most common at the age of 4. During the past 12 months, 47.7 % of children contracted more than two common colds, 9.5 % of children experienced more than one bout of acute otitis media, 6.9 % had more than one tonsillopharyngitis episode, and 3.2 % had rhinitis weekly or monthly.

In the present study we demonstrate that the number of visits due to URTI strongly correlated with the total number of visits, which indicates that visits due to URTIs have a strong impact on the direct costs of primary healthcare in children. This was not a surprise since visits due to respiratory infections were responsible for about 25–40 % of the total number of visits to primary care establishments in our study (median number of all visits was 32, median of all visits due to respiratory tract infections was 13, and median of visits due to URTI was 8). We also found that the age of the first visit due to URTI correlated with the annual indicator of visits due to URTI. Similarly, the age of the first antibiotic treatment correlated with the annual indicator of visits due to respiratory tract infections. The older the child at the time of the first antibiotic treatment, the less frequent the visits due to a respiratory tract infection were made. We think that the child's age at the time of the first visit as well as the age at the time of the first antibiotic treatment may be useful prognostic factors. This finding may be explained by reduced personal or environmental predisposition of a child to URTIs (e.g., not attending kindergarten) or by a higher parental threshold for visiting a pediatrician.

The most important finding of our study is that the overuse of antibiotics may result in an increase in the total number of visits and the number of visits due to URTI. We found a strong positive correlation between the indicator of annual overall antibiotic therapies and specifically therapies due to URTI and the indicator for visits due to URTI. Similarly, the annual indicator of antibiotic therapies due to URTI correlates with that for the total number of visits to the healthcare establishment. These correlations do not imply a causal relationship, but along with the results of previous large-scale studies by Otters et al. (2004) and Fleming et al. (2003) a conclusion can be drawn that a reduction in unnecessary antibiotic treatment may decrease the number of visits due to URTI. Otters et al. (2004) found, in a Dutch national survey, that decreasing antibiotic prescription rates in children paralleled a decline in the incidence of respiratory tract infections in general practice. Fleming et al. (2003) showed that a decrease in antibiotic prescription rates is directly related to a decrease in respiratory infections presented in general practice. Other explanations, like a decrease in the occurrence of URTIs in the general pediatric population or an increase in the threshold for consulting a pediatrician, are less likely because, in our study, we compared the same population of children over the same time. Another possible explanation of this relationship may relate to the influence of parents; those with a lower threshold for consulting doctors may be the same parents who insist on antibiotic treatment.

We demonstrate that antibiotics are overused and misused in respiratory infections in children in Poland. The majority (57.4 %) of respiratory infections were treated with antibiotics. The most common non-specific URTIs were treated with antibiotics in 25.8 % of cases. The situation in Poland is similar to that observed in other countries. Antibiotic use is generally highest among children and about 70 % of all antibiotics are prescribed for URTIs in children (Finkelstein et al. 2000; Majeed and Moser 1999). In our study, acute tonsillitis was treated with antibiotics in 90.7 % of cases, bronchitis in 67.5 %, otitis media in 65.9 %, pneumonia in 60.9 %, laryngitis in 22.2 %, and sinusitis in 12.5 %. The overuse and misuse of antibiotics contribute to the development of resistant bacteria and have been a major worldwide concern for many years (Neu 1992). Results of studies conducted at the end of the twentieth century indicated that Polish primary care doctors over-use antibiotics in acute pharyngitis and URTIs (Windak et al. 1996; Touw-Otten and Johansen 1992). In our study, 84.6 % of children were treated with antibiotics and the first ever antibiotic was prescribed due to respiratory tract infections in 65.9 % of them. The most common misuse of antibiotics is the treatment of viral infections (Mainous et al. 2003; Vaccheri et al. 2000; Nyquist et al. 1998; McCaig and Hughes 1995).

With the exception of acute tonsillitis, where streptococcal etiology is frequent, and in a fraction of acute otitis media and acute rhinosinusitis, where bacteria play a role, the vast majority of URTIs in children are viral. Acute pharyngitis is often the first symptom of an upper respiratory tract infection. Acute otitis media is the most common condition for which antibiotics are prescribed for children in the U.S., although viruses were detected in most cases, with or without bacterial involvement. Virus RNA was detected in 75 % children (35 % rhinovirus, 28 % RSV, 17 % coronavirus, and 5 % dual) (Pitkäranta et al. 1998). In addition, middle ear effusion is commonly found in colds, but may not be clinically significant (Bollag et al. 1996). Uncomplicated rhinosinusitis, without clinically evident extension of the inflammation outside the paranasal sinuses and nasal cavity, usually accompanies common colds. Although acute bacterial sinusitis is a relatively frequent complication of viral URTI or allergic inflammation, it possibly affects only 6–7 % of children requiring medical care for respiratory symptoms (Wald et al. 2013).

The weakness of our study is a limited number of patients and the fact that the data were collected from a single primary healthcare establishment, where the personal preferences of a few doctors could strongly influence the results. To minimize the aforementioned negative impact we selected a large primary care practice characterized by a high turnover (i.e., many doctors working for short periods of time). Another weakness of our study is that diagnoses were based on clinical symptoms; however, that is a rule in general practice (Hryniewicz et al. 2010; Heikkinen and Järvinen 2003). Our study was retrospective with all the inherent biases, but the results are, in our opinion, more reliable than those originating from questionnaires.

5   Conclusions

Respiratory tract infections in children are responsible for a substantial amount of primary healthcare visits in Poland and antibiotics are overused in their treatment. Education about the rational antibiotic use and implementation of careful and responsible management of a rational use of antibiotics is urgently needed since reducing the number of antibiotic therapies may decrease the number of visits due to upper respiratory tract infections as well as the total number of primary healthcare visits.

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Impact of Updated European Consensus Guidelines on the Management of Neonatal Respiratory Distress Syndrome on Clinical Outcome of Preterm Infants

S. Smolarova, L. Kocvarova, K. Matasova, M. Zibolen, and A. Calkovska

Abstract

European Consensus Guidelines (ECG) on the management of respiratory distress syndrome (RDS) have been developed and updated twice since 2007 reflecting changes in practice as new evidence emerges. The aim of this study was to evaluate the progress in clinical outcome of babies after the implementation of the updated ECG in 2010. Forty-eight neonates born in 2002–2003 (Group 02/03; n = 15) and in 2012–2013 (Group 12/13; n = 33) at gestational age of 26.2 ± 1.7 weeks were included into this retrospective study. Resuscitation procedures, ventilation support, and postnatal administration of surfactant were assessed. In Group 12/13, compared with Group 02/03, there was a higher rate of maternal corticosteroid prophylactic treatment (33 % *vs*. 0 %, p < 0.001), more children received primary nasal continuous positive airway pressure (nCPAP) (54.5 % *vs*. 20 %, p < 0.01) and repeated doses of surfactant (33 % *vs*. 0 %, p < 0.001), and had a reduced rate of mortality, bronchopulmonary dysplasia, and necrotizing enterocolitis. We conclude that the management of extremely preterm newborns improved considerably over the decade resulting in a significant reduction of mortality and morbidity.

Keywords

Maternal corticosteroids • Neonatal respiratory distress syndrome • Pulmonary ventilation • Surfactant replacement

S. Smolarova (✉) and A. Calkovska
Department of Physiology, Jessenius Faculty of Medicine, Comenius University, 4 Mala Hora St., Martin 036 01, Slovakia
e-mail: smolarova@gmail.com

L. Kocvarova, K. Matasova, and M. Zibolen
Clinic of Neonatology, Jessenius Faculty of Medicine, Comenius University, Martin and University Hospital, Martin, Slovakia

1   Introduction

Neonatal respiratory distress syndrome (RDS) is a condition of pulmonary insufficiency that commences at or shortly after birth and increases in severity over the first 2 days of life. If left untreated, death can occur from progressive

hypoxia and respiratory failure. In survivors, resolution begins between 2 and 4 days (EuroNeoStat 2006). The disease correlates with structural and functional lung immaturity, mainly caused by lack of pulmonary surfactant, which prevents alveolar collapse at the end-expiration. The incidence increases with decreasing gestation, with the EuroNeoStat Annual Report for Very Low Gestational Age Infants (2006) figures showing the prevalence of 92 % at 24–25 weeks', 88 % at 26–27 weeks', 76 % at 28–29 weeks', and 57 % at 30–31 weeks' gestation.

Clinically, RDS presents with early respiratory distress comprising cyanosis, grunting, retractions, nasal flaring, rapid or shallow breathing, unusual breathing movements, or other typical distress symptoms that usually occur in a premature infant immediately after birth, although they may not be seen for several hours.

The aim of the management of RDS is to provide interventions that will maximize the number of survivors while minimising the potential adverse effects. Over the past 40 years, many strategies and therapies for prevention and treatment of RDS have been developed and tested in clinical trials. Many of these have been subjected to systematic reviews and recommendations for the management of RDS have been created. The European recommendations for the treatment of newborns with RDS were first published in 2007 (Sweet et al. 2007) and since then they were reviewed twice. These recommendations are created on evidence-based practice, with the main aim to unite and to use optimal therapeutic procedures.

The aim of the present study was to evaluate the clinical outcome of neonates managed according to the updated European Consensus Guidelines on the management of respiratory distress syndrome in 2010, compared with babies who were cared for without any standardized recommendations in the past.

2 Methods

The study was approved by a local Ethical Committee of Jessenius Faculty of Medicine in Martin, Slovakia (permit no. EK/1187/2012).

Forty-eight preterm neonates born in years 2002–2003 and 2012–2013 at the Clinic of Neonatology, JFM CU and University Hospital Martin, Slovakia were included into a retrospective study. The babies were divided into two groups with no differences in gestational age and birth weight. Neonates born in 2002–2003 were included in Group 02/03 (n = 15; gestational age 26.3 ± 1.6; birth weight 849.7 ± 169.8 g) and those born in 2012–2013 made up Group 12/13 (n = 33; gestational age 26.2 ± 1.8; birth weight 905.3 ± 262.9 g). Initial management of preterm neonates, including type and duration of ventilation, early administration of pulmonary surfactant, and the need for its repeated doses, was followed in each group on the basis of medical records. Prenatal care, especially administration of corticosteroids to the mother with the risk of pre-term labor, was also monitored. Early postnatal course of late complications after preterm delivery such as bronchopulmonary dysplasia, necrotizing enterocolitis, and retinopathy of prematurity, and mortality rate were evaluated.

Categorical data were tabulated and evaluated with the chi-square test using Yates's correction. Other data were expressed as means $\pm$ SD and were evaluated with a t-test. $P < 0.05$ was regarded as statistically significant. Statistical analyses were performed with Statistica CZ ver. 10.

3 Results

Not a single mother of a child from Group 02/03 completed corticosteroid treatment (0 %). In Group 12/13, in almost one third of the mothers corticosteroid therapy was completed (n = 10; 30.3 %) (p < 0.001).

Data on surfactant replacement therapy are summarized in Table 1. Early administration of pulmonary surfactant at the delivery room, or within 2 h after birth, was performed in 10 infants (67.0 %) from the Group 02/03. No child required repetitive dose of surfactant in this group. In Group 12/13, pulmonary surfactant was given to 25 infants (75.8 %) and repeated dose was required by 11 of them (33.3 %). The maximum number of doses was three.

Table 1 Timing and repetitive administration of pulmonary surfactant

		2002–2003		2012–2013	
		Number of babies	Percent	Number of babies	Percent
Early administration of surfactant	Yes	10	67.0	25	75.8
	No	5	33.0	8	24.2
Repeated administration of surfactant	Yes	0	0	11	33.3
	No	0	0	22	66.7
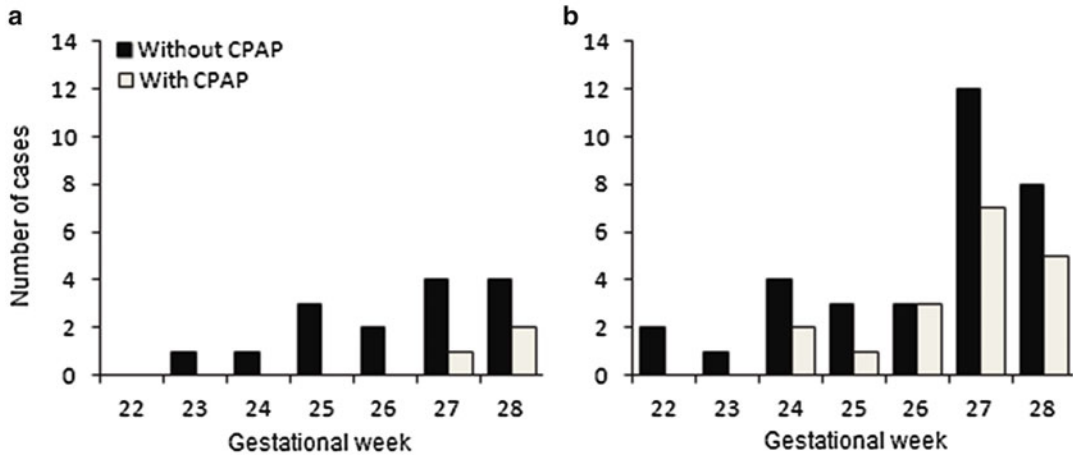
Fig. 1 Number of neonates with primary ventilatory support by nasal CPAP born in 2002–2003 (*Panel A*) and in 2012–2013 (*Panel B*)

In Group 02/03, three premature infants (20.0 %) were primarily managed with nasal continuous positive airway pressure (nCPAP) (Fig. 1a). After 2 h on nCPAP, no child from this group had mechanical support of ventilation. In Group 12/13, on the other hand, 18 children (54.5 %) were stabilized with CPAP and 9 infants (33.3 %) required intubation and mechanical ventilation after 2 h on nCPAP (Fig. 1b).

In Group (02/03), 78.0 % of children suffered from a late respiratory complication of bronchopulmonary dysplasia (n = 7); whereas this pathology occurred in 66.7 % (n = 18) of children in Group (12/13) (p > 0.05). Necrotizing enterocolitis (NEC) occurred in 2 neonates (13.0 %) in Group 02/03 with the duration of parenteral nutrition was 25.9 ± 15.4 days (median 21 days) in this group. In Group 12/13, necrotizing enterocolitis was present only in one patient (3.0 %), although the parenteral nutrition was administered only for 16.4 ± 6.9 days (median 14.5 days).

Table 2 Incidence of retinopathy of prematurity (ROP)

	2002–2003		2012–2013	
	Number of babies	Percent	Number of babies	Percent
With ROP	3	20.0	9	27.3
Without ROP	12	80.0	24	72.7

Retinopathy of prematurity (ROP) occurred in 20.0 % (n = 3) of children in Group 02/03, whereas it was recorded in 27.3 % (n = 9) of newborn infants in Goup 12/13 (Table 2). Mortality in Group 02/03 was 40.0 % (n = 6) and that in Group 12/13 was 27.0 % (n = 9) (p < 0.05) (Fig. 2a, b, respectively).

4 Discussion

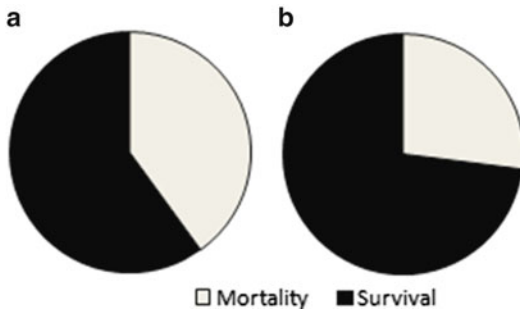Despite recent advances in the perinatal management of neonatal respiratory distress syndrome

Fig. 2 Mortality rate in neonates born in 2002–2003 (*Panel A*) and in 2012–2013 (*Panel B*)

controversies still exist. The aim of updated recommendations is to unite and start to use optimal therapeutic procedures, and to improve quality of care of preterm infants. The present study demonstrates that after the introduction of the European recommendations on the management of neonatal respiratory distress syndrome in preterm infants, clinical outcome of these newborns significantly improved over a decade resulting in a reduction of mortality and morbidity.

Several obstetric and neonatal procedures used in the management of children weighing 501–1,500 g changed in the years 2000–2009. In particular, a wider use of less invasive approach to ventilation has become more common (Soll et al. 2013). Early use of nCPAP led to decreased oxygen demand during the first 10 min, reduced a need for intubation, and shortened the time on mechanical ventilation and hospitalization. Moreover, quality of care of very immature children improved at the delivery room. However, a question of how to achieve a full implementation of the European guidelines on the management of respiratory distress syndrome in all hospitals and workplaces remains open.

4.1 Prenatal Care

Interventions to prevent RDS should begin before birth and involve both pediatricians and obstetricians as part of the perinatal team (Hansen et al. 2008). Mothers at high risk of preterm birth should be transferred to perinatal centers

experienced in the management of RDS. On the basis of the number of neonates in our groups we can say that there was an improvement in the antenatal care centralization. A greater number of infants in Group 12/13 reflects an increased number of pregnancy admissions *in utero*.

Preterm delivery can be delayed by using antibiotics in case of pre-labor rupture of the membranes (Kenyon et al. 2003), and tocolytic drugs can be used in the short-term to delay birth to allow a safe transfer to a perinatal center and to enable antenatal steroids to take effect. In our group we can confirm the improvement on corticosteroid treatment in mothers with pre-term labor; over a third of mothers received a full corticosteroid treatment. It is well established that women with threatened preterm delivery from about 23 to 35 weeks' gestation should receive a single course of antenatal steroids. Clinical studies have demonstrated that corticosteroids administered to mother reduce the risk of neonatal death and one completed treatment has no adverse effects on the mother or child. Antenatal steroids support maturation of the fetal lungs before birth and reduce the risk of developing RDS and its complications (Kuk et al. 2013). This effect is evident only in children whose mothers received the first dose of corticosteroids at the time of 1–7 days before delivery (Roberts and Dalziel 2006). To accelerate fetal lung maturation betamethasone or dexamethasone are used most commonly. Recent Cochrane Review suggests less intraventricular hemorrhage with dexamethasone. However, at present there are no clearly defined criteria regarding the choice of steroids (Brownfoot et al. 2008).

4.2 Mode of Ventilation

In the last update of the European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants, a new chapter is dedicated to non-invasive ventilation. Continuous positive airway pressure ventilation is an essential part of any strategy of non-invasive ventilatory support (Hillman and

Jobe 2013). It is a suitable alternative as an initial form of treatment even for extremely premature infants. Children weighing <1,000 g with gestational age <25 weeks can breathe and thrive on CPAP (Finer 2006). Nasal CPAP is non-invasive and it reduces lung injury and inflammation, improves oxygenation and reduces the need for mechanical ventilation (Bahareh et al. 2011). In spontaneously breathing babies the initial stabilization should start with nCPAP and PEEP of at least 5–6 cm H_2O *via* a mask or nasal prongs. During CPAP, excessive tidal volumes are avoided while at the same time PEEP is maintained in the airways. Intubation should be reserved for babies who have not responded to positive pressure ventilation or those who require surfactant therapy (Sweet et al. 2007, 2010, 2013). In Group 02/03 of the present study, before the introduction of the European consensus guidelines, only 20 % of preterm newborns were primarily stabilized with CPAP. After a decade, we saw a significant improvement in the initial stabilization, when more than a half of the babies were primarily managed with CPAP.

4.3 Surfactant Treatment

Prophylactic and early surfactant replacement therapy reduces pulmonary complications and mortality in ventilated infants with respiratory distress syndrome (Tsakalidis et al. 2012). In the first edition of the European guidelines of 2007, the prophylactic use of surfactant (within 15 min of birth) was recommended to almost all babies born at 26 weeks' gestation and to all preterm babies with RDS who require intubation for stabilization. However, prophylactic use of surfactant is not recommended in the last update of the European consensus guidelines of 2013; surfactant should only be administered as an early rescue treatment.

In the present study, 67 % of children in Group 02/03 received surfactant within the first two hours mostly as a prophylactic measure. In Group 12/13, on the other hand, 75.8 % of newborn infants received surfactant within two hours, but it was not a prophylactic measure. Surfactant was given after a period of nCPAP ventilation if the demand for oxygen still rose or clinical symptoms of RDS persisted. According to the current evidence, early CPAP and therapeutic administration of surfactant at the delivery room are the method of choice (Sweet et al. 2013). Early rescue surfactant should be administered to previously untreated babies if there is evidence of RDS. In babies who require surfactant, mechanical ventilation can be avoided by using the 'INSURE' (INtubate – SURfactant – Extubate to CPAP) technique and this method has been shown in randomized trials to reduce the need for mechanical ventilation and the subsequent development of bronchopulmonary dysplasia (Bohlin et al. 2008; Stevens et al. 2007). Recent studies indicate that another 'gentle' method for surfactant administration, Less Invasive Surfactant Administration (LISA) – an approach of keeping premature neonates on spontaneous breathing with continuous positive airway pressure support and administering surfactant by laryngoscopy *via* a small diameter tube – may even be superior to INSURE (Herting 2013).

5 Conclusions

After the implementation of the evidence-based guidelines on the management of preterm infants with RDS to the daily practice, the initial care of very preterm babies has become much less invasive. Moreover, the mortality rate and the long term outcome of the babies have improved significantly.

Conflict of Interests The authors declare no conflict of interests in association with this study.

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