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INFECTIONS IN CANCER CHEMOTHERAPY

A Symposium held at the Institute Jules Bordet, Brussels, Belgium

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PROTOCOL FOR AN INTERNATIONAL PROSPECTIVE TRIAL OF INITIAL THERAPY REGIMENS IN NEUTROPENIC PATIENTS WITH MALIGNANT DISEASE

H. Gaya*[1], J. Klastersky*[2], S. C. Schimpff*[3], M. H. N. Tattersall*[4].

INTRODUCTION

INFECTION remains a major cause of death in patients suffering from malignant disease, especially malignant blood disease, despite advances in cancer chemotherapy and supportive care in recent years. Many of these patients are profoundly neutropenic, and there is a quantitative relationship between the degree of neutropenia and the risk of severe infection. As well as predisposing to infection, neutropenia impairs the host's ability to overcome established infection, since circulating neutrophils constitute a defence against bacterial invasion and may increase the efficacy of some antibacterial agents.

Pyrexia is often the first and perhaps only warning of the onset of an infection which may lead rapidly to death, and infections in these patients are commonly caused by endogenous organisms although these may have been previously acquired by the host in the hospital environment. Because of the rapidity with which death may supervene it is important that antibiotic therapy is commenced as soon as infection is suspected and before the condition of the patient deteriorates to a point where therapy is unlikely to be successful. The only delay permissible is that necessary for the *taking* of blood cultures and any other relevant bacteriological specimens. In the absence of satisfactory rapid diagnostic methods one cannot afford to wait for laboratory results before instituting initial empirical antimicrobial therapy.

It is important to recognize that there are two distinct stages in the therapy of presumed infection in patients with neutropenia and malignant disease:

(i) The *first stage* consists of immediate empirical antibacterial therapy with a broad spectrum combination of antibiotics and continues until the results of the bacteriological cultures are known and the antibiotic susceptibilities of the isolated pathogens determined. It should not last longer than two or three days.

(ii) In the *second stage* inappropriate antibiotics are deleted from the initial combination and appropriate agents substituted or added to provide *specific* therapy for the infection.

Various two-, three-, four- and five- antibiotic combinations have been used in small trials in which the results of therapy are unimpressive and the numbers of patients studied have been far too small to be significant. Therefore in order to determine which antibiotic combinations are least toxic and most effective for initial therapy in febrile neutropenic patients with malignant disease the E.O.R.T.C. International Antimicrobial Therapy Project Group was formed. Membership of the group now extends to more than 20 centres throughout Europe and the U.S.A., and about 600 patients per year are being randomised to the treatment groups described below.

AIMS OF THE STUDY

(a) To define the incidence and types of infection which occur in neutropenic cancer patients.

(b) To evaluate, in a randomized prospective trial, the efficacy and toxicities of three two-antibiotic combinations used for initial empirical therapy in febrile neutropenic cancer patients.

(c) To determine whether a penicillin (or cephalosporin), or an aminoglycoside, or their possibly synergistic combination is more effective as specific therapy for the most commonly isolated pathogens.

SELECTION OF PATIENTS

All patients with malignant disease or marrow aplasia and neutropenia ($\leqslant$ 1000 neutrophils / μl) and with fever $\geqslant 38°C$ in the absence of obvious non-infective causes, *e.g.* blood product, L-asparaginase, cytosine arabinoside aministration, are eligible.

[1] St. Mary's Hospital Medical School, London, W2 1PG, Great Britain
[2] Institute Jules Bordet, Service de Médecine et Laboratoire d'Investigation Clinique, 1 rue Heger Bordet, Bruxelles, Belgium
[3] Baltimore Cancer Research Center, Baltimore, Maryland, U.S.A.
[4] Charing Cross Hospital, London, Great Britain
* for the E.O.R.T.C. International Antimicrobial Therapy Project Group.

Patients with renal insufficiency (serum creatinine > 2 mg %), and patients with known severe allergy to one of the antibiotics in the allocated regimen are excluded. All patients eligible for the trial but who are excluded for any reason whatsoever must be notified to the Co-ordinating Centre.

RANDOMIZATION

Patients are allocated to their treatment group (X, Y or Z) by drawing the next envelope from the stock supplied to each Participating Centre. The required patient details should be entered immediately on the post-card provided and sent to the Co-ordinating Centre, even if the patient is subsequently excluded from the trial.

containers:

(a) sterile dry tube

(b) lithium heparin tube.

The serum from (a) should be separated and deep-frozen. The plasma and red cells from (b) are separated and stored deep-frozen. These procedures should be repeated immediately before the next dose of antibiotic is given.

Thus, six specimens will be available –

2 specimens of serum (peak and trough)
2 specimens of red cells.
2 specimens of plasma.

INVESTIGATIONS

(i) *Mandatory*

	Before treatment	During treatment	One week after
Bacteriological			
Blood cultures	3 sets if possible	+	+
SWABS from possible foci of infection	+	+	+
URINE	+	+	+
SPUTUM	+	+	+
Virological	Acute serum for serology	Serum	Serum
Biochemical			
Serum creatinine and/or urea	+	+	+
Electrolytes (Na^+ and K^+)	+	+	+
Haematological			
Haematocrit	+	+	+
Haemoglobin	+	+	+
Total white cells	+	+	+
Differential white cell count	+	+	+
Thrombocytes	+	+	+
Clinical observations	Photocopies of patient's temperature chart during the period of study. Temperature to be recorded at least every 8 hr.		

(ii) *Optional additional investigations*

(a) On day 2 of treatment, 15 min after the end of administration of the antibiotic combination, 20 ml of blood should be taken and divided between 2

1. Serum specimens should be titrated against a light inoculum (10^4 organisms) of the infecting pathogen to determine the minimum bacteristatic and bactericidal dilutions.

2. The red cells should be lysed and the potassium concentrations in the plasma and lysate assayed [1].

(b) The minimum bacteristatic and bactericidal concentrations of the antibiotics in the trial regimen should be determined for the infecting pathogen.

(c) Serum levels of the administered antibiotics (peak and trough) should be estimated from the specimens taken for Optional Investigation A.1.

TREATMENT

(i) *Antibiotic regimens*

X – Carbenicillin + Cephalothin
Y – Carbenicillin + Gentamicin
Z – Cephalothin + Gentamicin

(ii) *Dosage*

For average adult, give:-

Carbenicillin: 24 g/m^2/day	Carbenicillin: 10 g/6-hr
Cephalothin: 7 /m^2/day	Cephalothin: 3 g/6-hr
Gentamicin: 180 mg/m^2/day	Gentamicin: 80 mg/6-hr

(iii) *Paediatric dosage*

The volume of water to be used for administration of the trial antibiotics should be calculated to give a similar concentration of each agent to that which is obtained using normal adult dosage dissolved in 100 ml water.

(iv) *Administration*

One quarter of the total daily dose of each antibiotic is dissolved separately in 100 ml water for injection and administered via a side-arm to an i.v. line over 30 min each 8 hr.

(v) *Treatment of infection*

1. Begin therapy with the allocated regimen and continue for at least 72 hr.

2. *Positive bacteriological results obtained:*

(a) If cultures yield	After 72 hr regimens are modified to:–		
	X	Y	Z
P. aeruginosa	Carb.	Carb. + Gent.	Gent.
Enterobacter	Carb.	Carb. + Gent.	Gent.
E. coli	Ceph.	Gent.	Ceph. + Gent.
Klebsiella	Ceph.	Gent.	Ceph. + Gent.

(b) After 72 hr of treatment, inappropriate antibiotics (on the basis of sensitivity tests) may be *stopped.*

(c) If other organisms are isolated, and/or pathogen-resistant to the antibiotics in the trial regimen, *add* antibiotic(s) indicated by sensitivity tests if not already being given.

3. *No bacteria isolated:*

(a) *Response to treatment* – Continue regimen for at least 5 days after temperature has returned to normal.
(b) *No response after 72 hr* – Other agents (e.g. antifungals) may be added to the regimen if clinically indicated, but the trial regimen *must be continued until 5 days* treatment has been completed.

(vi) *Optional additional measures e.g. White cell (granulocyte) transfusion, oral non-absorbed antibiotics, isolation.*

If any of these measures are used, they must be applied equally and without preference to each treatment group in the same way and for the same length of time.

(vii) *Antibacterial activity of the trial antibiotics (alone and in combination)*

	Cephalothin	Gentamicin	Carbenicillin	Carb. + Ceph.	Carb. + Gent.	Ceph. + Gent.
Clostridia	+		(+)	(+)	(+)	+
Streptococci	+		(+)	+	+	+
Staphylococci	+	+	±	+	+	+
Enterococci	(+)	x	(+)	(+)	+	+
Neisseria	+	+	(+)	+	+	+
Haemophils	(+)	+	(+)	(+)	+	+
Escherichia	+	+	+	+	+	+
Proteus	±	+	±	+	+	+
Klebsiella	+	+	+	+	+	
Enterobacter	±	+	±	±	+	+
Pseudomonas		+	+	+	+	+
Bacteroides	(+)		(+)	(+)	(+)	(+)
Mycobacteria						
Candida						
Other fungi		No useful activity				
Viruses		against these groups				
Rickettsia						
Protozoa						

+ Fully sensitive.
(+) Adequate activity in high dosage used.
± Only some species fully sensitive.
x Active only in synergic combination with another drug.

EVALUATION OF THERAPY

(i) *Classification of infections*

Microbiologically documented infection: Signs and symptoms of infection present and positive bacteriological cultures obtained.

Clinically documented infection: Site of infection identified and progress consistent with infection. Negative cultures.

Possible infection: Signs, symptoms and progress are consistent with infection. Negative cultures and no site known.

Doubted infection: Infection seems improbable on review of clinical signs and progress.

(ii) *Assessment of results of therapy*

Success: Lasting return of temperature, signs and symptoms to normal or to pre-infectious state.

Temporary improvement: As for "Success" but with relapse in 3–6 days despite continuing antibiotic therapy.

Failure: Infection persists and patient dies or is treated with other antibiotics.

No evaluation possible: Patient improves or remains unchanged but response cannot be related specifically to antibiotic therapy.

(iii) *Reporting*

Clinical report forms should be completed and sent to the Co-ordinating Centre *within two weeks* of completion of treatment. In addition to the results of mandatory and optional investigations, an attempt should be made to record in the appropriate sections the following data:

(a) Organisms causing Superinfection and/or Asymptomatic colonisation.

(b) Results of tests of hepatic function.

STATISTICAL ANALYSIS

At the end of one year the Clinical Report Forms will be analysed at the Co-ordinating Centre in accordance with the Aims of the Study.

PUBLICATIONS AND LECTURES

Nothing shall be published and no lectures shall be given by any of the participating members of the Project Group about either the work of the Group or the results of the Trial without consent of the Whole Group. However, each participant is free to publish data derived from his own patients and wards.

FURTHER INFORMATION

Persons wishing additional information about the Group should write to the Chairman & Trial Co-ordinator:

Dr. H. Gaya
Bacteriology Department
Wright-Fleming Institute
St. Mary's Hospital Medical School
London W2 1PG
England.

or, alternatively, to any of the other authors who are Joint Secretaries of the Group.

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PROSPECTIVE RANDOMLY CONTROLLED TRIAL OF THREE ANTIBIOTIC COMBINATIONS FOR EMPIRICAL THERAPY OF SUSPECTED SEPSIS IN NEUTROPENIC CANCER PATIENTS.*†

H. Gaya[1], J. Klastersky[2], S. C. Schimpff[3], D. Fière[4], S. Widmaier[5] and G. Nagel[6].

The preliminary results of a pilot trial of initial empirical antibiotic therapy for febrile episodes in neutropenic cancer patients are described. Infection was proven in 52% of the episodes.

Responses were excellent for all three antitiotic combinations. Overall, 88% of infections improved, as did 63% of the infections with associated bacteraemia.

So far we have inadequate data to judge the relative efficacy of the three combinations described with respect to specific bacteria. Therefore, we cannot comment on whether a cell wall active agent or an aminoglycoside, or their possibly synergistic combination, is superior.

Superinfections, subsequent infections and toxicities were acceptably low for each regimen.

INTRODUCTION

NEUTROPENIC patients with cancer have a high frequency of severe infections, especially those caused by gram-negative bacilli such as *Pseudomonas aeruginosa* or Klebsiella. Rapid progression to "septicaemia" with a two- or three- day median survival has led to the recognition of the need for prompt institution of empirical antibiotic therapy. It is not yet clear which antibiotic or combination of antibiotics should be chosen for such initial blind therapy [1–4].

The need for controlled clinical trials is obvious but single institutions do not have the requisite number of patients for large scale investigations. With these problems in mind, a group of investigators from oncology centres in Europe and the United States, under the auspices of the European Organisation for Research on the Treatment of Cancer (E.O.R.T.C.), devised a common protocol for inter-centre use. It is described in this Symposium [5].

The institutions included in this initial trial were centres in England, Belgium, the United States, Germany, France and Switzerland. Many other institutions had planned to enter the trial but because of the energy crisis following the 1973 Middle East war, there was a marked and unexpected shortage of ticarcillin and it was decided therefore, that the trial would end and be reinstituted using carbenicillin in place of ticarcillin. The new trial has started and about 20 centres are now participating. This report concerns only 124 cases entered under the original protocol.

MATERIAL AND METHODS

The present study was designed to define further the infections that occur in neutropenic cancer patients and to evaluate, in a randomised prospective trial, various logical combinations of agents for initial empirical therapy.

In addition, the testing of whether a penicillin-type drug, or an aminoglycoside, or their possibly synergistic combination is more effective against the most commonly isolated pathogens has been planned. For example, would the penicillin analogue ticarcillin alone, or the aminoglycoside gentamicin alone, or their combination be the most efficacious against *Pseudomonas aeruginosa*? Similarly, would cephalothin alone or gentamicin alone, or their combination, be most efficacious against Klebsiella? Additional objectives were to assess the frequency of both superinfections and subsequent infections, and to observe for toxicities.

[1] St. Mary's Hospital Medical School, London, England.
[2] Institut Jules Bordet, Bruxelles, Belgium.
[3] Baltimore Cancer Research Center, Baltimore, U.S.A.
[4] Hôpital Edouard Herriot, Lyon, France.
[5] Evangelische Krankenhaus, Essen-Werden, West Germany.
[6] Kantonsspital, Basel, Switzerland.

* E.O.R.T.C. International Antimicrobial Therapy Project Group.

† On behalf of the Group, the authors thank the Beecham, Schering and Eli Lilly Companies for their generous support.

Empirical therapy was instituted promptly for patients with malignant disease, neutropenia $\leqslant 1000/\mu l$, a temperature $\geqslant 38°C$ and a serum creatinine level < 2 mg/100 ml. Patients were excluded if an infecting organism was already known or if recent blood products or chemotherapy could reasonably explain the rise in temperature. The minimum pre-randomisation diagnostic investigations included history, examination, urine analysis, chest X-ray, blood cultures and cultures of relevant sites. Randomisation determined which of three combination regimens the patient was to receive: either ticarcillin + cephalothin, or ticarcillin + gentamicin, or cephalothin + gentamicin. Each combination has one or both agents active against all of the pathogens which most commonly infect this patient population. The antibiotics were given in the combinations and dosages illustrated in Table 1; each was administered separately by rapid i.v. infusion every six hours.

Table 1. Antimicrobial regimens used

Randomization to:		
Ticarcillin + Cephalothin	Ticarcillin + Gentamicin	Cephalothin + Gentamicin
Dosage:		
Ticarcillin	12 g/m^2/day	(~5g/6 hr i.v.)
Cephalothin	7 g/m^2/day	(~3g/6 hr i.v.)
Gentamicin	180 mg/m^2/day	(~80mg/6 hr i.v.)

The procedure for adding, deleting or changing antibiotics in relation to time depended on the patient's progress and the data that became available with regard to the presence or absence of infection. The complete definitions of infection and those of response or failure are documented elsewhere in this Symposium [5]. In a microbiologically documented infection, *i.e.*, if a specific site and specific bacteria were documented, antibiotics were deleted from the original combination depending on the organisms isolated. For example, if *Pseudomonas aeruginosa* was isolated as the sole infecting agent, patients receiving the combination of ticarcillin and cephalothin would be continued only on ticarcillin. In the second regimen, both antibiotics, possibly synergistic, would be continued, and in the third group only the gentamicin would be continued. For Klebsiella sp. only cephalothin would be continued in the first regimen, gentamicin in the second, and the possibly synergistic combination of cephalothin and gentamicin would be continued in the third.

Clinically documented infections refer to those instances where a site, but no organism is known. These patients had both original drugs continued. Neither additions nor deletions were allowed unless the patient left the protocol by reason of failure. A third section was classified as possible infection because although no site or organism was defined the patient appeared septic at initiation of antibiotics and had a prompt improvement in temporal relation to the administration of the antibiotic combination.

A febrile episode was classified as infection doubted and all antibiotics were discontinued if no infection could be documented within a reasonable length of time, *e.g.*, 4–5 days. Finally, the trial was considered non-evaluable and the antibiotic combination was discontinued if the patient proved to have a non-bacterial infection.

RESULTS

The classification of the 124 patient trials is shown in Table 2 and indicates that bacteraemia was subsequently detected in 13 %, and other microbiologically-documented infections in 18 % of the trials. Clinically-documented infections accounted for 21 % and possible infections for 10 %. Infection was ultimately ruled doubtful in 29 %, and 10 % were non-evaluable because of viral or fungal infections or protocol violations.

Table 2. Classification of patient trials

Bacteraemias	16 (13 %)
Microbiologically documented*	22 (18 %)
Clinically documented	26 (21 %)
Possible	12 (10 %)
Doubted	36 (29 %)
Non evaluable†	12 (10 %)
Total	124 (100 %)

* Minus bacteraemias

† Includes fungal and viral infections, protocol violations, etc.

The response rates for each antibiotic regimen and for all three combined are listed in Table 3 for each category of infection classification. For patients with bacteraemias the responses are essentially the same in the three regimens with an average improvement of 63 %. The patients who had non-bacteraemic microbiologically documented infections fared considerably better with 95 % improving. Likewise, the patients with clinically documented infections had a 92 % improvement rate and those with possible infections, by definition, all improved. The overall improvement rate for the 76 evaluable cases is 88 %.

Table 3. Response

	TC	TG	CG	Total
Bacteraemia	3/5*	4/6	3/5	10/16 (63 %)
Microbiologically documented†	6/6	9/10	6/6	21/22 (95 %)
Clinically documented	6/7	8/9	10/10	24/26 (92 %)
Possible	4/4	2/2	6/6	12/12 (100 %)
Total evaluable cases	19/22	23/27	25/27	67/76 (88 %)

*Improved/Total
†Minus bacteraemias
TC: Ticarcillin + Cephalothin
TG: Ticarcillin + Gentamicin
CG: Cephalothin + Gentamicin

Table 4. Response

Organism	Improved/Total
Escherichia coli	10/11 (91 %)
Pseudomonas aeruginosa	7/7 (100 %)
Staphylococcus aureus	7/7 (100 %)
Klebsiella spp	2/4 (50 %)
Other bacteria	5/9 (56 %)
Total	31/38 (82 %)

The most frequently isolated bacteria are listed in Table 4. The overall improvement rate is given because there are no differences at present between the three regimens. The responses with *E. coli, Ps. aeruginosa* and *Staph. aureus* were excellent. Two of four patients, however, died with Klebsiella sepsis. The occurrence of side effects and superinfections is summarized in Table 5.

Table 5. Toxicity and superinfection

Regimens used*	TC	TG	CG
Total patients trials	36	41	47
Toxicities			
Plebitis	1	3	4
Hypokalaemia	3	1	0
Azotaemia	0	0	2
Anaphylaxis	1	0	0
Rash	1	0	0
Superinfection and Subsequent infection†	8	5	7

*TC = Ticarcillin + Cephalothin
TG: Ticarcillin + Gentamicin
CG: Cephalothin + Gentamicin
†Occurring within 1 week of completing antibiotics

DISCUSSION

We are, of course, gratified with these results and suspect that the overall success is attributable in large measure to the very early institution of antibiotic therapy [2]. We recognize, however, that 29 % of 124 cases never had an infection defined and, presumably, did not require the four days of broad spectrum antibiotics received.

Toxicities were fairly infrequent. Phlebitis occurred in each group, and hypokalaemia occurred only in the regimens including ticarcillin. Azotaemia was uncommon possibly because antibiotics were begun early before hypotension had occurred. However the number of cases is still small and other investigators have reported significant nephrotoxicity with the cephalothin–gentamicin combination [4]. Non-fatal anaphylaxis and skin rash occurred only once each. Superinfections and subsequent infections, though not uncommon, were of an acceptable frequency considering the population of patients at risk.

Responses were excellent for all three antibiotic combinations. Overall, 88 % of infections improved, as did 63 % of the infections with associated bacteraemia. Only further controlled trials, such as the one presented here, may in the future indicate the optimal therapeutic attitude towards the infected cancer patient.

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AGGRESSIVE CANCER TREATMENT AND ITS ROLE IN PREDISPOSING TO INFECTION

Martin. H. N. Tattersall
Department of Medical Oncology, Charing Cross Hospital, Fulham Palace Road, London, W6, Great Britain.

The effects of cancer treatment on host defences against infection are discussed. The main factors predisposing cancer patients to infection are identified as granulocytopenia, disturbed phagocyte function, immunosuppression, and breaches in mucosal and skin continuity. In addition, alteration of the normal microbial flora which may accompany hospitalization, antibiotic treatment, and cytotoxic chemotherapy and the risks of supportive care measures are considered. A number of approaches to reducing infection risks during cancer treatment are suggested.

SEVERE infection occurs frequently in cancer patients. The infections often end fatally and always limit what otherwise might be successful cancer treatment, [1, 2]. These infections are caused primarily by bacteria, but a variable proportion are due to fungi, viruses, higher bacteria and protozoa. The frequency and aetiology of infections occurring in cancer patients depend on the tumour type, and the tumour's effect on the bone marrow and on humoral and cellular immunity [3–5]. Many factors are known to affect host resistance to infection. In cancer patients, alterations of host defence mechanisms may exist before therapy is instituted [6–8]. The drugs used in cancer treatment, as well as surgery and radiotherapy also alter host defences, but the relative contributions of these factors in pre-

Table 1. Disturbances of normal host defences by cancer treatment

Host defence	Treatment
A. Protection afforded by normal flora	Cytotoxic drugs with antimicrobial activity Steriods Antibiotics Hospital admission
B. Anatomical barriers	Diagnostic and therapeutic measures: Surgery, Supportive care Cytotoxic drugs Corticosteroids Tumour necrosis
C. Inflammatory response	Radiotherapy Cancer chemotherapy Malnutrition Supportive care
D. Reticulo endothelial system	Radiotherapy Cancer Chemotherapy
E. Immune response	Cancer Chemotherapy Radiotherapy Surgery

disposing individual cancer patients to a risk of infection are difficult to assess [8–12].

Until more selective cancer treatments are available, it is inevitable that cancer patients will be exposed to substantial risk of infection when therapy of their primary disease is undertaken. A knowledge of the disturbance of host defence mechanisms caused by cancer treatment, and an understanding of the infection risk factors associated with cancer treatment may permit measures to be taken so that more aggressive cancer treatment may be given without concurrently increasing the risks of infection.

Table 1 lists individual host defence mechanisms against infection and indicates how they may be disturbed by cancer treatment. It can be seen that cancer treatment may disturb host resistance in several ways and it is frequently difficult to define the relative contribution of each compromised defence mechanism in the aetiology of a given infection. In general, it may be said that disturbance of granulocyte production and function is associated most clearly with bacterial and fungal infection, that a defect of humoral immunity is associated with bacterial and some viral infections, and that a disturbance of cellular immunity is associated with protozoan, chronic bacterial and some viral infections.

Granulocytopaenia

The single most important factor predisposing to bacterial infection in patients with malignant disease is a low granulocyte count. Infectious episodes in cancer patients are related to the degree and duration of granulocytopaenia. Bodey *et al.* have reported on the quantitative relationship of these factors to the occurrence of infection in leukaemic patients [13]. It has not been established that exactly the same relationships exist when granulocytopaenia is due to cytotoxic chemotherapy. A number of investigators have observed that relatively few serious infections occur in patients with chronic neutropaenia and Rebuck skin window studies in these patients have shown that the neutrophil responses as in leukaemia patients are roughly proportional to the blood neutrophil count, but that mononuclear cell accumulation is usually normal [14]. Patients myelosuppressed by cytotoxic drugs usually have both neutropaenia and monocytopaenia and thus they may be more at risk of infection than patients with chronic neutropaenia. Recently Deinhard *et al.* [15] have reported studies on the neutropaenia of cancer chemotherapy; they concluded that chemotherapy may be continued safely despite peripheral neutropaenia as long as the bone marrow reserve and tissue leucocyte inflammatory responses are intact. This finding suggests that no clear quantitative correlation between peripheral neutrophils and the occurrence of infection in cancer patients may exist when the neutropaenia is due to myelosuppressive treatment. Radiotherapy may contribute to neutropaenia in cancer patients particularly when the area of marrow irradiated is large, and granulocyte function may also be impaired (see below).

Supportive therapy in cancer patients may precipitate granulocytopaenia. Prolonged granulocytopaenia accompanying transfusion of HLA incompatible platelet concentrates has been described recently in two febrile patients with aplastic anaemia [16], and Brittingham and Chaplin observed earlier the phenomenon of acute transient neutropaenia associated with transfusions of leucocyte rich blood fractions in patients with prior histories of febrile transfusion reaction [17]. Similar observations in volunteers transfused with aliquots of ABO incompatible red blood cells have been reported [18]. In cancer patients with compromised bone marrow reserves, the neutropaenia which may follow incompatible transfusions of red cells, leucocytes or platelets may be particularly severe and prolonged, and thus extreme care is necessary in these patients to ensure that transfused cells are appropriately matched.

How can granulocytopaenia due to cytotoxic therapy be minimized? Pending the availability of more selective cancer treatment, a number of approaches designed to enhance anti-tumour effect while sparing host toxicity may be followed. Firstly, there are approaches designed to achieve high tumour cytotoxic drug concentrations with lower levels in normal tissues. Regional perfusion techniques and topical application are possible only with a minority of tumours, but approaches using antibody as a drug carrier [19] or liposome encapsulation so that only endocytic tumours will concentrate the drugs are being developed [20]. Selective cytotoxic drug activation by enzymes contained in tumour but not normal cells is another approach which is being explored [21]. A second approach is that of selective rescue. It has been demonstrated in experimental tumours and in some human tumours, that high dose methotrexate–folinic acid rescue has an enhanced therapeutic index compared to methotrexate alone [22–24]. Although the exact reasons for this enhanced selectivity are not known, it is possible that this principle of 'rescue' may be appropriate with other drugs. Thymidine enhances the therapeutic index of methotrexate in some experimental tumours, and thymidine mouthwashes may minimise the mucosal toxicity of methotrexate in man [25]. Thus, selective tumour toxicity may be possible using techniques which rescue normal tissues. A third

approach is to modify tumour or bone marrow kinetics so that the drugs are given at the time when tumour cells are particularly sensitive to attack. A number of investigators are attempting to selectively recruit tumour cells into drug sensitive phases of the cell cycle, and bone marrow toxicity may be reduced by hypertransfusion [26, 27]. Finally the duration of granulocytopenia following myelosuppressive drugs may be reduced by androgen therapy [28]. The optimal timing and effectiveness of this treatment in cancer patients receiving cytotoxic myelosuppressive treatment has not been determined nor has the effect on infection risk been demonstrated.

Impaired granulocyte function

While granulocytopaenia is certainly important, the role of qualitative defects in phagocyte function in predisposing cancer patients to infection may also be important. Phagocytosis and killing of bacteria is a function of cells derived from the bone marrow. Neutrophil polymorphonuclear leucocytes, monocytes and tissue macrophages comprise the phagocytic system. There are two types of macrophages: fixed cells which include liver and spleen macrophages, and free macrophages such as those in the alveolar spaces, which are migrating phagocytes. Each type originates from blood monocytes and in the process of differentiation, their phagocytic and digestive function are modified. However, in general, inherited or acquired defects of one component of the phagocytic system are mirrored in the whole system. The development of sensitive and specific *in vivo* and *in vitro* assays of phagocytic function, has led to the recognition of specific functional defects of phagocytosis in patients with increased risk of infection, and changes in these functions following cancer treatment have also been identified. These tests permit greater definition of enhanced susceptibility to bacterial infection of cancer patients and suggest ways in which these infection risks may be minimised.

Functional disorders of phagocytosis can be divided conveniently into four groups: chemotaxis and migration, opsonisation, ingestion and intracellular bacterial killing [29]. *Chemotaxis* is a remarkable characteristic of phagocytic cells which describes their capacity to be attracted by certain chemical substances and micro-organisms and to move in a straight line towards the attracting agent. Chemotaxis therefore depends upon recognition of the chemical attractant, and this is determined by the presence or absence of appropriate receptors on the phagocytic cell surface. In addition various serum factors have been found in patients with lymphoproliferative diseases which inhibit phagocyte chemotaxis and recently it has been shown that the tissue ascorbic acid level may influence phagocyte chemotaxis [30]. Another factor which may influence the ability of leucocytes to respond to chemotactic factors is the neutrophil stickiness. Decreased neutrophil stickiness has been reported in acute and chronic granulocyte leukaemia and drugs, notably ethanol, aspirin and prednisone have been noted to interfere with granulocyte adherence [31, 32]. The effects of other drugs are being studied. The vinca alkaloids have been shown to impair neutrophil chemotaxis but not their random migration and these findings suggest that cell surface receptors may be affected [33].

Clearly, chemotaxis depends on the ability of phagocytes to move. The isolation of actin and myosin from leucocytes has heightened interest in the role of these proteins in phagocyte movement [34]. Recently a reduction in phagocyte chemotaxis has been noted during hyperalimentation and it appears that this defect is due to hypophosphataemia which reduces phagocytic ATP levels, and disturbs energy supplies for phagocyte movement [35]. Thus, a number of drugs and metabolic disturbances affect phagocyte migration and chemotaxis (Table 2).

Opsonization is a term given to the coating of bacteria with serum factors which alter their physicochemical characteristics to enhance phagocytosis. Both heat stable and heat labile serum factors are important [29]. The former are IgG antibodies directed against specific antigens of the bacterial surface. The heat labile serum factors are components of the complement system. Both direct antibody-mediated complement activation and the alternative pathway of complement activation generate on opsonically active fragment of C3 on the surface bacteria. Abnormalities of the complement system are known to exist in a number of inherited and acquired diseases states characterized by an increased susceptibility to bacterial infection and it is also known that high dose steroids and some cytotoxic drugs may disturb the synthesis of complement components and thereby disturb opsonization [36]. Moreover, most anti-cancer drugs are also immunosuppressive and thus disturb specific antibody synthesis [8]. Low serum opsonic activity may be seen in patients receiving cancer treatment and this indicates a possible role of antibody prophylaxis against specific microbial antigens in these patients.

Ingestion, which is a function of mature phagocytes, depends on the presence of specific receptors on the phagocyte surface and is also an active energy dependent event. Vinblastine and colchicine have been reported to disturb phagocyte ingestion, possibly by reducing cellular deformability as a result of microtubular precipitation [37]. Hypo-

Table 2. Cancer treatment and phagocyte production and function

Factor	Treatment
Decrease production	Radiotherapy Myelosuppressive drugs
Decrease mobilisation and chemotaxis	Steroids Vinca alkaloids/colchicine Hyperalimentation Hyperglycaemia
Decrease opsonic activity	Steroids Cytotoxic drugs
Impair phagocytosis	Vinca alkaloids/colchicine Hyperalimentation Morphine analogs Hyperglycaemia
Reduce bactericidal reactions	Vinca alkaloids/colchicine Steroids Hyperalimentation Craniospinal irradiation Sulphonamides
Impair reticulo-endothelial function (Fixed macrophage function)	Cyclophosphamide, 5-Fluorouracil Radiotherapy Steroids

phosphataemia which may accompany hyperalimentation has also been associated with reduced phagocyte ingestion perhaps due to a lowering of intracellular ATP level [35]. Hyperglycaemia which may follow high dose steroid treatment also impairs phagocytosis, but the mechanism has not been defined [38].

The final stage of phagocyte function involves a number of *intracellular reactions* which kill the ingested microbe. The various stages in this process may be separated into the creation of a phagocytic vacuole, degranulation of cytoplasmic lysosomal granules into the phagocytic vacuole, stimulation of phagocytic oxidative metabolism and finally the death and digestion within the phagocytic vacuole of the ingested organism. Defective phagocyte microbicidal activity has been observed in a number of inherited and acquired conditions. Cancer treatment may also impair phagocyte microbicidal activity and this has been reported following systemic steroid treatment in rats and in man [39, 40]. Alcohol and colchicine impair microbicidal reactions *in vitro* [41, 42], but the importance of this effect *in vivo* is not known. Hyperalimentation associated with hypophosphataemia [35] and craniospinal irradiation impair phagocyte microbicidal activity *in vivo* [43], but irradiation of granulocytes *in vitro* does not [44]. Sulphonamides at high concentration *in vitro* impair phagocyte microbicidal reactions, but it is not known if this occurs *in vivo* [45]. Thus, cancer treatment may disturb the final stage of phagocyte function and the possibility that such damaged phagocytes may protect intracellular bacteria from the effects of antibiotics which cannot penetrate phagocytes may be an additional problem [46].

Effects of corticosteroids

The number of drugs which are known to influence phagocyte function is not large, and the importance of this aspect of impairment of host defence mechanisms is not known. As can be seen in Tables 1–3, the adrenocorticoids are the group of drugs used most widely in cancer patients which disturb white cell function. The bulk of the evidence from both animal and human studies suggests that the steroid effect on leucocyte mobilisation is particularly important in the drug-induced impairment of host resistance to bacterial and fungal infection, but clearly the effect on phagocytosis and microbicidal reactions compound the impairment of host defence mechanisms [38, 40, 47, 48]. Recent studies of neutrophil kinetics in patients receiving alternate day and daily prednisone indicate that, on the day off prednisone, neutrophil kinetics return toward normal and this may explain the lack of dramatic susceptibility to infection in patients on this drug

schedule [49]. The possibility that steroid administration which is used widely to increase the yield of neutrophils from granulocyte donors, might carry a "steroid" lesion has been raised by Cline [50]. The recent studies of Shafi and Vogler indicate that hydrocortisone administered to the donor increases the granulocyte yield without impairing their phagocytic or bactericidal activity, but no studies of their *in vivo* distribution or half lives have been reported [51]. The earlier observation that dexamethasone, unlike prednisone, does not impair localized leucocyte mobilization in man indicates that seroids may differ in their effects on host defence mechanisms [47].

Table 3. Drugs and host defence mechanisms

CORTICOSTEROIDS

1. Impair phagocyte mobilisation to inflammatory site
   - –decrease responses to chemotactic stimuli
   - –decrease neutrophil stickiness
2. Impair phagocytosis
   - –decrease specific antibody synthesis causing impaired opsonisation
   - –raise blood glucose which reduces phagocytosis
3. Impair microbicidal activity
   - –alter neutrophil metabolic response to ingestion
   - –impair discharge of lysosomal enzymes
4. Impair reticuloendothelial activity
   - –reduce spleen and liver macrophage function
5. Depress antibody formation
   - –lympholytic action
   - –decrease immunoglobulin synthesis
6. Depress T cell function
   - –suppress PPD reactivity, and lymphocyte response to mitogens
7. Alter the gastrointestinal flora
8. Cause direct tissue toxicity, enhance tissue invasion and dissemination of fungi
9. Depress formation and activity of interferon

Reticulo-endothelial system

The importance of fixed tissue macrophage phagocytic activity (reticulo-endothelial system) in the containment of infection in man is not known. The increased frequency of fatal septicaemia in splenectomized children may not be due only to a decreased bulk of reticulo-endothelial tissue. Adults, with Hodkin's disease in whom staging splenectomy has been carried out, probably have an increased incidence of septicaemia, but the mortality is not greater than in non-splenectomised patients [52]. Studies of the clearance of labelled aggregated albumen, or labelled bacteria have indicated that cytotoxic drugs and radiotherapy generally depress clearance [53, 54]. Clearance of endotoxin from the circulation may be an important function of the reticuloendothelial system, and increased plasma endotoxin clearance rates following induction of endotoxin tolerance have been correlated with markedly reduced morbidity and mortality in experimental animals following endotoxin challenge [55]. Thus, it may be that compromised reticulo-endothelial activity may be responsible for the high mortality rate from gram negative infections in cancer patients.

Immune suppression

As already mentioned, resistance to infection involves the action of phagocytic cells, antibody production, and the activity of lymphoid cells. The majority of studies of the effects of cancer treatment on host defence mechanisms have been concerned with the effects on antibody production, delayed hypersensitivity and allograft rejection. The extent of immune suppression varies with the agent and the length of administration. Cyclophosphamide, and the nucleic acid antimetabolites 6-mercaptopurine, 5-fluorouracil and methotrexate reduce antibody response to foreign antigens but some other cytotoxic drugs have no effect [8]. The mechanisms of action of anti-cancer drugs determine the type of immune disturbance which results. A drug which inhibits cellular proliferation has greater effects on a primary than a secondary immune because cellular proliferation is less prominent in the secondary response. Corticosteroids, alkylating agents, and intercalating antibiotics used in cancer treatment, have somewhat different effects. In general, cytotoxic treatment is most immunosuppressive when antigen is administered shortly before chemotherapy. The time to recovery of immune function after one dose of a cytotoxic drug is usually 2–3 days but this varies with the drug pharmacology. Following prolonged drug treatment, several weeks may be required for recovery of immune responses.

It is recognized that B Lymphocytes are associated with the production of immunoglobulins, while T Lymphocytes are associated with delayed hypersensitivity and blastogenic responses to mitogens. The bulk of anti-cancer drugs apart from steroids depress B cell function more than T cell function. Studies of changes in T cell function in cancer patients receiving combination chemotherapy have demonstrated a relationship between rebound and overshoot of T cell function and tumour response [11]. The effects of chemotherapy on cytotoxic B lymphocytes are more prolonged, but since these lymphocytes may participate in tumour rejection,

intermittent high dose cytotoxic treatment is preferred in tumour treatment, while chronic low dose administration is more usual in the field of transplant control, and autoimmune disease. The effects of radiotherapy on immune functions have been studied in detail and prolonged immune disturbances following treatment have been described [56]. Lymphopaenia, with a specific loss of T cells, has been described following craniospinal and mammary irradiation [10, 57]. An immunosuppressive effect of surgery has also been reported [12].

The contribution of lymphopaenia to the incidence of infection in cancer patients is less certain than that of granulocytopaenia, but it does appear to be related to the occurrence of viral and fungal infections.

The types of infection occurring in immunosuppressed patients have been related to the type of immunosuppressive treatment and the specific features of host defences which are impaired [3]. The importance of specific antibody in resistance to infection has been established for pseudomonas and certain viral infections [58, 59]. In the former case, the importance of local or secretory antibody in the respiratory tract has been stressed [60]. Trials of polyvalent pseudomonas vaccine have established the value of this prophylaxis in the management of severely burned patients but the results in cancer patients, and patients with cystic fibrosis have been less clear [61, 62]. Clinical observation in virus infection complicating immunodeficiency states has indicated that antibody appears to prevent viral spread to distant organs. Viral antibodies not only can inactivate extracellular virus but also may decrease virus production through effects on virus infected cells. A clinical association between more frequent and severe herpes or pox viral disease and compromised cell mediated immunity has also been reported [59]. The importance of cancer treatment in predisposing patients to viral infection is not known, but immunosuppressive treatment in bone marrow transplant patients has been accompanied by a substantial mortality from viral infections [63].

There is increasing evidence that immunosuppressive chemotherapy in cancer patients and renal transplant patients may cause activation of latent viruses, and thus be the basis for the occurrence of multifocal leukoencephalopathy in immunosuppressed patients. Cytomegalovirus and EB virus also appear to be capable of latent infection of white blood cells with activation to more extensive infection during immunosuppression.

Interferon which is produced by cells in response to viruses and other intracellular parasites, limits the spread of infection to adjacent cells. The increased frequency of viral disease in cancer patients may be related to impaired interferon response. The effects of different cancer treatment on interferon responses have not been studied extensively nor have studies of interferon inducers in cancer patients been completed.

A high frequency of pneumocystis infection has been reported in patients with neoplastic disease, particularly in those with malignant lymphoma and leukaemia [64–66]. The occurrence of this infection in leukaemia patients in remission has been described but the immune defect or factor which predisposes cancer patients to infection with this organism has not been defined. However, experimentally induced pneumocystis pneumonia in laboratory animals can be influenced by certain drugs used in cancer patients. Chlorambucil, cyclophosphamide, methotrexate and steroid treatment have been reported to influence the development of active pneumocystis infection in these animals [67].

The association between leucopaenia and fungal infection has been described in several reports [68, 69], and in one study multiple organ candidiasis was significantly more frequent in granulocytopaenic patients [68]. In contrast, hypogammaglobulinaemia does not appear to be an important predisposing factor to fungal infection. Irradiation of laboratory animals increases their susceptibility to fungal infections, and although this may be related to the degree of granulocytopaenia, it seems possible that other factors such as disturbed phagocyte function may also contribute [70]. The importance of corticosteroid treatment in predisposing to fungal infection has been suggested and experiments in laboratory animals support these observations [71, 72]. The mechanisms of this effect have not been defined, but the association between Hodgkin's disease and cryptococcal meningitis which was noted prior to the use of cytotoxic drugs indicates that impairment of delayed hypersensitivity may influence the development of fungal infection [73].

BCG immunotherapy may have a role in the treatment of a number of malignant diseases, but already some complications of this treatment have been reported. Some cancer patients receiving BCG immunotherapy with cytotoxic chemotherapy, have developed an infection with this organism which has caused hepatic dysfunction (in some patients) [74, 75]. The administration of viable organisms as immunotherapy for patients with compromised defence mechanisms is clearly a risky undertaking; it is important to establish that such treatment is superior to treatment with killed organisms.

Mucosal Barriers

Mucosal continuity is particularly important in preventing the entry of pathogens in patients with compromised phagocytic and lymphoid cell function. As has been emphasized, the basis of present-day cancer treatment is cell destruction and normal cells are affected as well as neoplastic ones. The destruction of cells lining the gastrointestinal tract, urinary tract and respiratory passages by cytotoxic drugs is undoubtedly a major factor in the penetration of organisms into the host. Cytotoxic chemotherapy may also alter gastrointestinal cellular function and lead to malabsorption syndrome and malnutrition, and permit entry of endotoxin from the bowel. The relevance of endotoxin to the morbidity and mortality of bacterial infection during cytotoxic chemotherapy is unknown.

Non-specific infection risks

In addition to the effects on host defence mechanisms of specific cancer treatment, cancer patients are exposed to further infection risks due to hospitalization, supportive care, minor surgical procedures and sophisticated investigations. Alterations of superficial bacterial flora have been noted in severely ill patients [76] with a change towards Gram-negative colonization [77]. Schimpff and his colleagues have found that most infections in acute leukaemia are caused by hospital-acquired organisms [78, 79]. Organisms may be acquired from many sources in hospital, including food and water [80], humidifiers and respirators [81], flowers [82] and patient-to-patient transfer. The reasons why it is primarily "ill" patients in hospitals that become colonized with hospital organisms is not known. There are several reasons for Gram-negative organisms being particularly common in hospital. It has been recognised that some disinfectants favour growth of Gram-negative organisms and that antibiotic and cytotoxic chemotherapy may predispose to Gram-negative colonization [83]. The latter observation is based on the anti-bacterial spectrum of many anti-cancer drugs. Metcalfe observed that beta haemolytic streptococci were isolated very frequently from patients with acute leukaemia receiving cytotoxic chemotherapy [84]. The anti-bacterial activity of methotrexate probably explains this observation, for streptococci are inhibited at concentrations reached in body fluids during methotrexate treatment. Bodey studied the antimicrobial activity of a number of cytotoxic drugs and noted that pseudomonas was particularly resistant to the drugs and suggested that the frequency of pseudomonas sepsis in patients receiving cytotoxic chemotherapy might be due to selection of pseudomonas by repeated cytotoxic chemotherapy [85].

The use of broad spectrum antibiotics alters the normal bacterial flora and may predispose to colonization by resistant organisms. The occurrence of superficial fungal infection in patients on these antibiotics has been described frequently and in cancer patients such infection may disseminate rapidly.

Many hospital-based procedures expose cancer patients to increased risks of infection. Sigmoidoscopy [86], urinary catheterization [87] and intravenous infusions [88] facilitate the entry of organisms which in cancer patients may not be cleared by already compromised defence mechanisms. The administration of potentially infected parenteral fluids such as platelet concentrates stored at room temperature and some batches of commercial intravenous fluids may result in septicaemia in cancer patients [88]. Intravenous catheters may be an important portal of entry particularly for candida infections, and the frequency of fungal septicaemia has been related to the time an i.v. line has been in place [89, 90]. Surgery in cancer patients may add to the impairment of host defence mechanisms [12] as may malnutrition [91] and hyperalimentation when it is accompanied by hypophosphataemia [35].

Cancer treatment thus impairs host defence mechanisms against infection in many ways. In addition, it may interefere with diagnostic measures which are taken when infection is suspected. Cytotoxic drugs may impair the growth of organisms in blood cultures, and thus delay diagnosis of bacteraemia, they may lead to positive limulus tests due to their effect on the gut mucosa, and they may complicate the interpretation of the NBT test due to direct effects on granulocyte function. Cancer patients due to both their primary disease and to the effects of therapy frequently do not have normal inflammatory responses, and thus clinical signs of infection may be masked leading to further delay in diagnosis.

Experimental approaches

Pending the development of more selective treatment for cancer patients, it seems certain that the present era of aggressive therapy will be accompanied by substantial risks of infection. Elsewhere in this symposium, the value of protected environments, non-absorbed antibiotic prophylaxis and granulocyte transfusions in preventing infection in cancer patients are being discussed. In addition, I wish to re-emphasize some other approaches which may prove valuable in protecting patients receiving aggressive cancer treatment. The effect of androgen administration which may shorten the duration of granulocytopaenia following chemotherapy needs evaluating

in man since in experimental animals, granulocytopaenia following antimetabolite chemotherapy can be markedly reduced.

Another approach which merits study is the induction of endotoxin tolerance in cancer patients. In experimental animals it has been shown that repeated low dose administration of endotoxin induces a state of tolerance so that otherwise lethal doses of endotoxin are well tolerated. A programme in cancer patients is planned; we have noted that patients receiving non-absorbed antibiotics by mouth have an improved prognosis in documented septicaemia compared to patients not receiving this prophylaxis, and it is possible that the basis for this observation is that the antibiotics have depleted the quantity of endotoxin in the bowel and thus reduced the endotoxin load which may enter the systemic circulation during an infection. Lastly, the recognition that phagocytes with defective microbicidal reaction may protect ingested micro-organisms from antibiotics should encourage the synthesis of more lipidsoluble antibiotics or the use of antibiotics in lipsomes.

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CAUSES OF DEATH IN ACUTE NON-LYMPHOCYTIC LEUKEMIA

Jean Klastersky, Danièle Weerts and Claude Gompel

Service de Médecine Interne et Laboratoire d'Investigation Clinique (Section des Maladies Infectieuses) et Service d'Anatomie Pathologique, Institut Jules Bordet, 1000 Bruxelles, Belgique.

The causes of death were evaluated in 32 patients with acute non lymphocytic leukemia admitted consecutively to a special unit at the Institut Jules Bordet. Infection caused death in 13 (40·6 %) out of 32 patients. Most fatal infectious episodes were bacteremias caused by Gram-negative rods, most often *E. coli*. Hemorrhage also was responsible for the death of 13 (40·6 %) patients. The patients who ultimately died from infection had lived for a shorter period of time than those who died from other causes. The patients who died from infection had undergone strict isolation in laminar air flow rooms or gastrointestinal sterilization less often than the patients in the other group. The patients who died as a result of infection had evidence of more severe myelosuppression than those who died from other causes. This study emphasises the need for further progress in the prevention and management of infection in patients undergoing aggressive chemotherapy.

INTRODUCTION

RECENT improvements in the therapy of patients with cancer by new cytostatic agents and combination chemotherapy programs can alter significantly the natural history of several neoplastic diseases. Therefore, periodic evaluation of the causes of death in patients admitted to centers specialising in cancer therapy may provide guidelines relative to areas where further research on supportive care in malignant diseases is needed. In patients with leukemia, especially in those with acute non-lymphocytic leukemia (ANLL), infection and hemorrhage are considered by most authors as the commonest causes of death [1, 2]; however, a recent analysis of the causes of mortality in this type of patients is not available.

Our evaluation was undertaken therefore, to examine the causes of death in a series of patients with ANLL admitted consecutively to a special unit for the treatment of leukemia at the Institut Jules Bordet over a relatively short period of time. Special emphasis has been put on infection as a cause of death in these patients and a comparison between the characteristics of the patients who died as a result of infection and those who died from other causes has been attempted.

It should be noted that only overwhelming terminal events have been taken into consideration to decide which cause of death was involved in each case. Similarly, to evaluate the frequency of non-fatal infection, whether suspected or bacteriologically proven, and that of non-fatal hemorrhages, only major episodes have been taken into consideration.

Please address requests for reprints to Dr. J. Klastersky; Service Médecine, Institut Jules Bordet, rue Héger Bordet, 1000 Bruxelles, Belgium.

MATERIAL AND METHODS

The charts of 42 patients with ANLL admitted over a four-year period (June 1970 to June 1974) to a special unit at the Institut Jules Bordet were reviewed. In 10 patients, no adequate information regarding the cause of death could be determined since these patients died outside the hospital; these patients were not considered for the present study.

In the other patients, the clinical and pathological causes of death were analysed and the final cause of death determined. Only pathological states sufficient to cause death alone or in combination were recorded for that purpose, excluding minor degrees of infection, hemorrhage or other conditions.

Clinical signs and symptoms of severe infection were required to consider sepsis as a cause of death. Septicemia was considered as a cause of death if positive blood cultures were obtained during the last 10 days of life and/or positive heart blood was obtained at autopsy. For pneumonia, clinical features of a respiratory death were required as well as bacteriological or serological evidence of a pulmonary bacterial or viral pathogen. Disseminated fungal infection and fungal broncho-pneumonia were diagnosed at autopsy on tissue sections.

To be considered as a cause of death, hemorrhage had to be extensive or present in a vital organ. Death was attributed to leukemia itself in cases where extensive infiltration of a vital organ had occurred with corresponding premortem clinical evidence of failure of that organ.

Only treatment, given during the last hospital stay, was considered in the evaluation of the role of therapy in the causes of death. Full therapeutic doses had to be given commencing at least 1 week prior to death.

RESULTS

The present study concerns 32 patients with ANLL who died over a 4 year period at the Institut Jules Bordet after being hospitalized in the same special medical unit.

rooms) and had their gastrointestinal tracts sterilized for shorter periods of time than the patients who died from causes other than infection. The frequency of proven and suspected infectious episodes was slightly higher in the patients who ultimately died from infection; on the other hand, the number of hemorrhagic episodes was higher in the other group. These differences were small and of questionable significance.

As indicated in Table 2, infections were responsible for death in 13 (40·6 %) patients. Most were bacterial infections as will be described later. In these patients, bleeding into the central nervous system might have contributed to death in 4 cases and pulmonary involvement by hemorrhage or leukemia in 6 other patients. Hepatitis was found at autopsy in 2 patients and fibrosis of the lung, presumably due to busulfan administration, in 1; these

Table 1. Characteristics of the population studied

	Total	Mortality due to infection	Mortality due to non-infectious diseases
Number of patients	32	13	19
Diagnosis			
Acute myeloblastic leukemia	23	10	13
Acute monoblastic leukemia	4	0	4
Acute promyelocytic leukemia	3	1	2
Blastic transformation of chromic myelogenous leukemia	2	2	0
Total duration of disease(days) (p.p.)†	4825 (150·8)	1508 (116·0)	3317 (174·5)
Number of hospital stays (p.p.)	79 (2·4)	31 (2·3)	48 (2·5)
Duration of hospitalization(days) (p.p.)	1818 (57·1)	694 (53·4)	1124 (58·6)
Number of days in isolation (p.p.)	748 (23·3)	236 (18·1)	512 (26·9)
Number of days decontaminated (p.p.)	376 (11·7)	95 (7·3)	281 (14·7)
Number of proven infections (p.p.)	45 (1·3)	25 (1·8)	20 (1·05)
Number of suspected infections (p.p.)	19 (0·6)	10 (0·8)	9 (0·5)
Number of hemorrhages (p.p.)	73 (2·2)	26 (2·0)	47 (2·4)

† p.p. = per patient.

* 10 other patients with ANLL were hospitalized during the study period but the cause of death could not be documented in them.

Table 1 indicates the type of ANLL involved in these patients and other characteristics of the population studied. It can be seen that the total duration of the disease averaged 5 months and was shorter in the patients who died from infection than in those who died from other causes. Death usually occurred during the second stay of the patient in the hospital and the total duration of hospitalization was about 2 months. The patients who died from infection spent less time in complete isolation (in laminar flow

latter pathological findings were probably of little relevance to the fatal outcome.

Fatal hemorrhage occurred in 13 (40·6 %) patients and occurred in the digestive tract in 6 cases, in the central nervous system in 6 and in the lungs in 1. Bleeding at other sites was frequently associated with the major episode of bleeding in the gastrointestinal tract, central nervous system or the lung as well as with an important leukemic infiltration of

Table 2. Principal and associated causes of death in patients with ANLL

Principal causes of death		No. of fatal episodes	Associated causes of death: Infection		Hemorrhage			Other causes		
			fungal	viral	central nervous system	gastrointestinal tract	lungs	leukemia	hepatitis	busulfan lung
Infections	bacterial	9			4		2	4	1	
	fungal	3							1	1
	viral	1								
Hemorrhages	central nervous system	6				1	1	1		
	gastrointestinal tract	6			1		1	2		
	lungs	1	1		1	1		1		
Other causes	leukemia	3			1	1				
	cardiac failure	2								
	pulmonary infarct	1								

Table 3. Characteristics of fatal infections

No.	Type of infection (and source)	Microorganism	Days after admission	Duration prior death	Granulocytopenia	Adequate therapy	Leukemia present
1	Disseminated infection*	*C. albicans*	1	7	yes	yes	yes
2	Disseminated infection*	*C. albicans*	99	2	yes	no	no
3	Septicemia (urine)	*Serratia marcescens*	6	10	yes	yes	?
4	Septicemia (GI tract)	*E. coli*	27	3	yes	yes	no
5	Septicemia (GI tract)	*E. coli*	3	2	no	yes	yes
6	Septicemia (GI tract)	*E. coli*	12	2	no	no	?
7	Septicemia (?)	*E. coli*	8	10	yes	yes	no
8	Septicemia (?)	*E. coli*	1	2	no	no	no
9	Septicemia (?)	*E. coli*	40	6	yes	yes	yes
10	Septicemia (?)	Klebsiella sp.	77	16	yes	no	yes
11	Bronchopneumonia*	*C. albicans*	11	3	yes	yes	yes
12	Pneumonitis (viral)†	Respiratory synticial	6	12	yes	no	?
13	Perineal cellulitis‡	*P. mirabilis*	12	23	yes	yes	yes

* Demonstrated at autopsy.

† Rise in complement fixing antibodies against respiratory syncitial virus from 1/2 to 1/128 during the last 7 days of life.

‡ Heart's blood at autopsy as well as spleen tissue were positive for *P. mirabilis;* bacteremia was not documented prior to death.

various organs. It is difficult to determine the frequency of bacterial or viral infections that might have been associated with hemorrhage in these patients: many were febrile but definite evidence of severe infection could not be established in them, otherwise they would have been considered as having died from infection. A renal abscess, caused by *C. albicans,* was detected in a patient who died as a result of massive pulmonary hemorrhage. Massive leukemic infiltration of many organs, namely the lungs, heart and kidney was thought to be the cause of death in 3 patients. The present series is too small to allow the study of a possible relationship between the type of leukemia and the cause of death. Finally, 3 patients died of causes unrelated to infection, hemorrhage or leukemic infiltration of tissues: in 2 of them global cardiac failure was documented and in one case multiple large pulmonary infarcts were discovered at autopsy.

The types of fatal infections observed in this series are indicated in Table 3. Disseminated fungal infection (*C. albicans*) was seen in 2 patients at autopsy and probably originated from the urinary tract in one of them. Bacteremia, caused by Gram negative rods in all cases, was documented prior to death in 8 out of 13 (61 %) patients who died from sepsis and at autopsy in another patient with perineal cellulitis caused by *P. mirabilis;* thus, 9 out of 13 (69 %) patients who died from infection did so as a result of Gram-negative sepsis. *E. coli* was responsible for 6 episodes of septicemia; in 3 patients *E. coli* strains similar to those isolated from the blood were cultured from the stools; in the 3 other patients the source of infection could not be found. In one patient, septicemia caused by *Serratia marcescens* originated from the urinary-tract and in another septicemia, due to Klebsiella sp., no obvious focus could be demonstrated. Bronchopneumonia caused by *C. albicans* was demonstrated at autopsy in 1 patient and a diffuse pneumonitis caused by respiratory syncitial virus was documented in another patient on clinical and serological grounds.

As indicated in Table 4, fatal infections occurred later during the last admission than fatal hemorrhages or other fatal conditions. During the first week of admission there were 7 deaths among which only 1 was caused by infection; among the 14 deaths that occurred within the first 2 weeks after admission, only 5 were due to infection. The hemorrhagic episodes caused 4 and 7 deaths respectively in the patients who died within 1 and 2 weeks after the admission. Granulocytopenia was more frequent (18·6 days per patient) in patients who died from infection than in those who died from other causes (9·3 days); thrombocytopenia was also more frequent in the patients who died as a result of infection.

As could be expected, fever was found more frequently in patients who ultimately died from infection; as already mentioned, strict isolation was used more frequently during the last period of hospitalization in patients who died from causes other than infection.

Hemorrhage occurred with a similar frequency in both groups but transfusions of platelets were given

Table 4. Complications and therapy (per patient) during the last admission in patients who died in the hospital

	Mortality due to infection	Mortality due to non-infectious diseases
Number of patients	13	19
Duration of the final admission (days)	30·4	14·8
Duration of granulocytopenia* (days)	18·6	9·3
Duration of thrombocytopenia† (days)	12·1	7·1
Duration of fever‡ (days)	16·6	10·1
Duration of isolation (days)	1·8	7·9
Number of hemorrhages	2·5	2·8
Number of platelet transfusions	4·2	2·4
Number of proven infections	1·5	only 1 episode
Duration of antibiotherapy (days)	15·1	5·8
Number of granulocyte transfusion	0·8	0·3

*1000 granulocytes/mm^3 or less
†10,000 platelets/mm^3 or less
‡38°C or more

more often to the patients who presented fatal infections; this probably reflected the more severe thrombocytopenia in these patients.

Microbiologically-proven severe infections were more frequent in patients who died from sepsis; as a matter of fact only 1 episode of infection could be documented among the patients who ultimately died from causes other than infection. This explains that antibiotics were given less often to these patients than to those who died from infection. Since antibiotics were also given empirically to febrile granulopenic patients, the lower incidence of both fever and granulocytopenia in patients who died from other causes than sepsis might also explain the lower frequency of antibiotic treatments in these patients.

Granulocyte transfusions were given more often to patients who presented a fatal outcome related to sepsis; the increased incidence of neutropenia, of febrile episodes and of proven infections may explain this finding. The results obtained in our hospital with transfusion of granulocytes are discussed in detail elsewhere in this volume.

13 patients who died from infection had no demonstrable leukemia at autopsy but were in a state of extreme bone marrow depression. Only 1 out of 14 patients who died from causes other than infection and in whom a complete autopsy was available had no demonstrable leukemia; this patient died from a massive pulmonary embolism.

DISCUSSION

The present study is of course very limited as far as the number of patients is concerned; however, it has the advantage of having been performed in patients who were all taken care of by the same medical and nursing staff over a period of time during which the supportive care of patients with ANLL has been quite uniform in the unit, especially with respect to the use of antibiotics and protected environment, and to the indications and technique of platelet and granulocyte transfusions.

This study confirms observations made by others [1, 2] stressing the importance of infection as the leading cause of death in ANLL. As far as the frequency of infection in ANLL patients is concerned, our results are close to those reported recently from the Baltimore Cancer Research Center [3] in which the "sepsis frequency" (the total number of hospital days divided by the total number of septicemia episodes) was 80; in our study, there were 19 documented septicemias (17 bacterial and 2 fungal) observed during 1818 hospital days; the "sepsis frequency" in our study was thus 95.

Table 5. Cytostatic chemotherapy administered during the 3 weeks preceding death

Chemotherapy	Mortality due to infection	Mortality due to non-infectious diseases
No cytostatic therapy	4	8
ARAC (cytosine arabinoside)	2	1
Daunomycin	2	0
Thioguanine	0	1
5-Fluoro-uracil	0	1
ARAC + daunomycin	1	0
ARAC + VCR (vincristine)	1	0
ARAC + thioguanine	0	2
ARAC + methotrexate	0	1
VCR + prednisone	1	1
VCR + bleomycine	0	1
VCR + ARAC + cyclophosphamide	1	0
VCR + methotrexate + cyclophosphamide	0	1
VCR + ARAC + daunomycin + cyclophosphamide	1	2

Table 5 summarizes the type of cytostatic therapy which has been given to the patients studied in the present series. Clearly, there were too many forms of chemotherapy to allow any meaningful study of the possible influence of the type of treatment of ANLL on the cause of death. It should be pointed out, however that granulocytopenia and thrombocytopenia were more pronounced in the patients who ultimately died of infection, possibly indicating a more aggressive therapeutic approach in these patients. It should also be stressed that 4 of the

Infection and hemorrhage were each responsible, in our series, for 40.6 % of the fatal episodes. Only overwhelmingly fatal events were taken into consideration for this analysis and it should be emphasized that the incidence of non-fatal complications, which are usually associated with significant morbidity in patients with cancer, is not reflected here. These figures are higher than those obtained for infection and hemorrhage as a cause of death in patients with solid neoplasic tumors [4, 5]; in these latter patients a significant proportion dies as a result of the extension of the tumor itself to vital structures in addition to infection and hemorrhage.

Most fatal infections seen in this series – and by others [1–3] under similar conditions – were Gram-negative septicemias; this is in accordance with the general increase of Gram-negative infections in most modern hospitals [6] and has clear therapeutic implications. Treatment of infection, as soon as it is suspected, should be undertaken immediately and with an antibiotic regimen primarily directed against Gram-negative microorganisms. The rationale for the use of synergistic combinations of antibiotics under these conditions has been presented in detail elsewhere [7]; it is sufficient to say that there are strong suggestions that in the compromized host such combinations of antibiotics are clinically more effective than non-synergistic ones. The choice of the types of antimicrobial therapy to be used in septic patients with ANLL or other debilitating conditions must take into consideration the sensitivity of the microbial flora of each particular hospital which is mainly influenced by the local prescribing habits.

It is essential for the clinician to know the prevalent flora in a given hospital since the microorganisms of which it is composed will sooner or later colonize patients who are hospitalized. Bacterial colonization of patients susceptible to infection is a phenomenon of major clinical importance: in about 50 % of documented bacterial infections in ANLL, the pathogens had become part of the patients' resident flora after having been acquired from the hospital environment [8]. It should be remembered however that the sensitivity *in vitro* to drugs does not uniformly predict the clinical outcome of treated infections.

Fungal infections were found in 3 patients (23 % of the fatal infections) and viral pneumonia in only one. Fungal, viral and protozoan infections are found more often in immunodepressed patients such as those with lymphocytic leukemia in remission; Gram-negative sepsis is more common in neutropenic patients [9].

In this series, the patients who ultimately died from infection had lived for a shorter time than those who died from other causes. In a retrospective study, involving a modest number of observations, it is difficult to determine how significant this observation might be. Nevertheless, it suggests that prevention of infection and cure of established sepsis might prolong life in patients with ANLL; therefore, a further effort in this direction is fully justified.

As far as prevention of infection is concerned, it should be stressed that, in the present series, the patients who died from causes other than infection had undergone isolation in laminar air flow rooms and gastrointestinal sterilization more often than those in the other group. That these procedures might reduce significantly the frequency of infection in patients with ANLL undergoing cytostatic chemotherapy has been suggested by several studies as indicated in a recent review [10].

Another striking difference between the patients who died from infection and those who died from other causes was the more pronounced granulocytopenia and thrombocytopenia in the former. Whether this was related to differences in cytostatic therapy in these 2 groups of patients is unclear; but 4 out of 13 patients in the more myelosuppressed patients had no demonstrable leukemia at autopsy while in only 1 of 14 examined patients in the other group could leukemia not be demonstrated at autopsy.

At this point, we are reaching at the basic and complex problem of the therapy of patients with ANLL: cure of leukemia necessitates aggressive myelosuppressive treatment which has a major role in the predisposition to severe infection, especially Gram-negative sepsis.

Therefore, the hope for cure or sustained remissions in ANLL not only depends on more effective cytostatic therapy than that which is available today but also on a better prevention and a more active treatment of infections. The data presented here show that bacterial infection is the major cause of death in ANLL today, suggesting that a considerable effort should be made to prevent and more readily cure infections in these patients. Procedures intended to reduce the threat of infection in patients with ANLL such as the adequate use of new antibiotics, alone or in combination, the transfusion of granulocytes and the realization of a sterile environment should be further investigated and evaluated.

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DIAGNOSIS OF INFECTION IN PATIENTS WITH CANCER

Stephen C. Schimpff

Section of Medical Oncology, Baltimore Cancer Research Center, National Cancer Institute at the University of Maryland Hospital, Baltimore, MD 20014, USA

The practice of oncology requires that infections be promptly and appropriately managed, a task dependent upon an understanding of the principles of infection predisposition and presentation in a wide variety of clinical settings. Adult patients with acute leukemia and profound granulocytopenia tend to develop pneumonia, anorectal lesions, pharyngitis, and skin lesions (with frequent bacteremias) caused primarily by Gram-negative bacilli and *Staphylococcus aureus*. Infections with yeasts and fungi tend to occur later in these patients' courses. Although patients with lymphoma have few infections other than *Herpes zoster* initially, infections similar to those found in acute leukemia develop in the granulocytopenic patient with propressive or recurrent lymphoma. Immunologic dysfunction in these same patients apparently predisposes to *Cryptococcus, Listeria, Cytomegalovirus, Pneumocystis* and *Toxoplasma* infections. Patients with carcinomas have infections related to local lesions and obstructive phenomena while brain tumors predispose to aspiration pneumonia and urinary infection. In all patients, iatrogenic procedures and hospital acquisition of organisms are major predisposing factors. Reduced or altered inflammatory responses necessitate meticulously thorough, repeated evaluations. An appreciation of the clinical setting and the operative predisposing conditions will usually allow a prompt and accurate diagnosis.

INTRODUCTION

THE PREVENTION and therapy of infectious complications have become major responsibilities of oncologic practice but appropriate management depends on a firm understanding of the principles of infection development in various clinical settings.

It is our purpose to show that certain principles of infection causation (tumor type and status, therapy, other predisposing factors) and presentation (altered inflammatory response) operate in these patients, which if understood, will aid in prompt detection, documentation, and therapy. Although the more unusual opportunistic infections occur in oncologic patients most infections are due to Gram-negative bacillii, *Staphylococcus aureus, Candida albicans, Varicella-zoster,* and the hepatitis viruses. The difficulties in documenting the etiologic agent in certain sites (pneumonia), specific factors which tend to obscure the site and true nature of many serious infections (granulocytopenia and ano-rectal lesions), and various iatrogenic maneuvers which may (hyperalimentation) or may not (splenectomy) predispose to infection will be discussed.

Address reprint requests to: Stephen C. Schimpff, Baltimore Cancer Research Center, 22 South Greene Street, Baltimore, MD 21201, USA.

DISCUSSION

Acute leukemia in adults and chronic myelocytic leukemia (in blast crisis)

Predisposing factors. The most important predisposing factor to infection in these patients is granulocytopenia secondary to either the leukemia or its therapy. Both the frequency and severity of infection are inversely related to the absolute granulocyte level especially below 500 granulocytes/mm^3 [1]. Today's intensive drug therapy, requiring an average of 20–50 days to reach remission, produces a granulocyte level of $< 100/mm^3$ during 50 % of treatment time. [2] Usually the only resolution of granulocytopenia is through remission; indeed, the ultimate preventive measure for infection is a complete clinical and bone marrow remission.

Other important predisposing factors include diminished leukocyte migration, reduced opsonic activity, the occasional marked mucosal ulcerations following drug therapy, prostatic hypertrophy from leukemic infiltration (reversible with radiation therapy), and other forms of obstruction such as leukemic infiltrates occluding the eustachian tube or sinuses (leading to otitis or sinusitis). Some of the most important predisposing factors are those caused by the physician himself: indwelling urinary catheters cause not only localized infection but can lead

promptly to bacteremia in this patient population. Indwelling venous catheters are well known for their propensity to cause both local and systemic infection and should be banned from use in patients with leukemia. Hyperalimentation is associated with an increased incidence of infection, particularly *Candida* spp and group D *Streptococcus* spp. Other infusion therapy problems include contaminated i.v. equipment or bottles and even the prolonged (> 48 hr) use of butterfly needles at a single sight [3, 4].

Recent evidence clearly implicates hospital-acquired organisms (not the patient's endogenous microbial flora) as the major cause of serious infections in these patients. *Pseudomonas aeruginosa* is a case in point; among 48 patients with acute nonlymphocytic leukemia only 9 were colonized with *Pseudomonas aeruginosa* at the time of admission but 22 patients acquired the organism during their hospital stay. Fifteen (68 %) of these 22 patients became infected including 13 who had one or more septicemias. These hospital-acquired infections represented over two thirds of the *Pseudomonas* infections and 84% of the *Pseudomonas* bacteremias among these patients [4, 5].

Infections caused by *Staphylococcus aureus* have also been found to be hospital-acquired; adequate evidence for *Klebsiella* spp and *Escherichia coli* are lacking but it can be assumed that inhalational infections such as *Aspergillus* pneumonia are probably due to hospital acquired organisms [4].

Acquisition of hospital organisms is a significant and serious predisposing factor to infection among these patients and must be considered when designing preventive and therapeutic techniques. All of the possible routes of organism acquisition are not yet well defined but overcrowded wards, poor personnel hand and clothing hygiene, contaminated food and water and contaminated air or air handling systems can often be implicated.

Infection sites and organisms. Although infections may arise in unsuspected sites and be caused by unusual organisms, over 80 % are associated with 5 or 6 sites and an equal number of organisms. The sites are the lower respiratory tract, anorectal area, pharynx, skin (especially axilla and groin), urinary tract, and liver (hepatitis). Less frequent sites include esophagus, colon, and sinuses [4]. Direct organism access via intravascular catheters, contaminated transfusions or fluids depends on infusion policies [6–8]. The organisms are *Pseudomonas aeruginosa, Klebsiella* spp, *Escherichia coli, Staphylococcus aureus, Candida* spp, and the hepatitis viruses. Less common are other Gram-negative bacilli, group D *Streptococcus* spp, *Aspergillus* spp, and *Phycomycete* spp. *Streptococcus* (*Diplococcus*) *pneumoniae, Hemophilus* spp, and other streptococci rarely cause infection [3, 4, 9–11].

Pneumonia. These are usually caused by Gram-negative bacilli, especially *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella* spp plus *Staphylococcus aureus, Candida* spp, or *Aspergillus* spp; *pneumococcal* pneumonia is exceeding rare [3, 4, 12]. Although there may be an increasing frequency of *Phycomycete* (mucor) pneumonia it is relatively uncommon to find a pneumonia caused by *Cryptococcus neoformans, Cytomegalovirus, Toxoplasma gondii,* or *Pneumocystis carinii.* The development of pulmonary tuberculosis appears to depend upon the patient's prior exposure. It is not yet established whether many common viral respiratory pathogens cause pneumonia or may predispose to more severe bacterial or fungal pneumonias.

Pneumonias are not only the most frequent cause of serious infection but also the most frequent cause of infectious death [2, 4, 12]. Because of poor inflammatory response associated with granulocytopenia there are frequently little or no physical findings at first examination and the chest X-ray may be perfectly normal. However, some minimal physical findings usually become apparent in a few days and an infiltrate develops on the chest X-ray. It is therefore essential to repeat both examination and radiographs daily to ensure pneumonia detection.

Sputum production is usually minimal and nonpurulent, and is of little diagnostic value because almost all such patients have various Gram-negative bacilli in their pharynx. It is therefore usually necessary to do a transtracheal aspiration to obtain uncontaminated material; if aspiration nets insufficient material it may be necessary to perform either transtracheal selective bronchial brushing or a lung biopsy to establish an etiologic diagnosis [12, 13].

Ano-rectal lesions. Prior to the introduction of prophylactic oral nonabsorbable antibiotics, perianal and perirectal lesions and abscesses were common in patients with acute monocytic leukemia (nearly 60 % developed these lesions) or in acute myelomonocytic leukemia (30 %) but they were relatively uncommon in acute myelocytic leukemia (10 %) and acute lymphocytic leukemia (10 %). True abscesses are uncommon; most lesions are actually small fissures at the anal opening which are stretched and further torn with each bowel movement. Apparently, the force of bowel movements pushing bacteria against these seemingly minor lesions is sufficient to cause

bacteremia in certain patients (AMOL and AMML) with granulocytopenia [14]. Even the most minor appearing lesion can be the source of a bacteremia, usually *Pseudomonas aeruginosa* or less commonly, *Escherichia coli* or *Klebsiella pneumoniae.* In some patients a true abscess of sorts develops which may be small or extremely large, usually with a watery-like consistency containing many bacteria but few granulocytes. Culture of the fluid invariably reveals Gram-negative bacilli, Gram-positive cocci and multiple anaerobes; however, only *Pseudomonas aeruginosa, Klebsiella* spp or *E. coli* tend reach the blood stream.

These lesions are easy to overlook, leading to the unacceptable diagnosis of "primary bacteremia". However, most can indeed be detected from the very first day if one looks diligently for the small mucosal tear at the anal opening whether or not suggested by a history of increasing pain with defecation [14]. As with pneumonia, if no site of infection is found initially, it is not only pertinent but essential to re-examine the patient daily with careful history, anal examination, and digital rectal examination.

Skin lesions. These are often related to sites of iatrogenic trauma (venipuncture, bone marrow aspiration) or to areas of ecchymosis and bleeding. Minor axillary folliculitis following shaving and/or the use of occlusive antiperspirants may progress to large abscesses with blood stream dissemination. Another frequent area of skin infection is near the groin perhaps because of fecal contamination. Many skin lesions are caused by *Staphylococcus aureus* but Gram-negative bacilli are almost equally as frequent. For unclear reasons, periorbital cellulitis, usually due to *Pseudomonas aeruginosa,* is frequent in adult acute leukemia. *Varicella-zoster* infections are infrequent in this group of patients [15] but *Herpesvirus hominis* (simplex) causes frequent and often severe local lesions about the mouth; dissemination is rare in acute leukemia. Penile lesions, probably initiated by *H. hominis,* become superinfected with Gram-negative bacilli and may progress to bacteremia. The skin is also a secondary site of many infections, *e.g.,* ecythema gangrenosum of *Pseudomonas aeruginosa* infections, [16] the papular lesions of disseminated candidiasis [17] or the various lesions of cryptococcosis [18].

Pharyngitis. This is a frequent complication following chemotherapy and may be related to the mucosal alterations caused by cytotoxic therapy. When bacterial or fungal pharyngitis occurs the patient usually complains of severe pharyngeal pain, difficulty swallowing, fever and occasional chills. The throat is erythematous but without exudate; there is no cervical adenopathy although the patient may complain of tenderness to palpation. Utilizing usual criteria, these cases of pharyngitis would appear to be remarkably benign but the poor inflammatory response must not preclude diagnosis because bacteremia from pharyngitis is not uncommon during profound granulocytopenia [4].

Throat cultures show multiple organisms such as Gram-negative bacilli, *Staphylococcus aureus,* and various yeasts but many of these episodes may actually be caused initially by viruses or mycoplasma. As in other sites, *Pseudomonas aeruginosa, Klebsiella* spp, and occasionally *Staphylococcus aureus* cause most bacteremias. *Candida albicans* and other *Candida* species are frequent causes of a disabling pharyngitis which may seriously limit swallowing, but most *Candida* septicemias probably arise from the esophagus or intestinal tract.

Sinusitis. During the last 5 years sinusitis has been an uncommon site for infection in adults with acute leukemia at the BCRC but recently has increased perhaps because of a move from a facility with circulating hot water to a new hospital with forced hot air which may subject the patients to reduced humidification. The etiologic agent is frequently very difficult to determine because nasal flora is often not representative of the affected sinuses. The need to know the agent is essential but invasive diagnostic procedures are hazardous because of associated thrombocytopenia. Most cases are probably viral in origin but *S. aureus* and Gram-negative bacilli are not uncommon. Although *Phycomycete* spp more commonly infect the lungs in leukemia, sinusitis must always invoke a suspicion of mucor mycosis which can be diagnosed with a nasal biopsy or occasionally with a wet preparation of a nasal smear [3, 11].

Esophagitis. The importance of this site was not fully recognized until recently when careful post-mortem analyses revealed that nearly 1/3 of adult patients with acute leukemia have esophagitis at the time of death, most frequently caused by species of *Candida* [19, 20]. Most cases originate in the lower third of the esophagus where a combination of cytotoxic related mucosal alterations and gastric acid reflex predispose to microbial invasion. A barium swallow at the first indication of burning substernal pain or dysphagia will often reveal a ragged mucosa [21]. Although *Candida* species are usually found at postmortem examination, barium swallow cannot identify the agent; early esophagoscopy with biopsy will frequently implicate *Herpesvirus hominis* or a Gram-negative bacillus such as *Pseudomonas aeruginosa.*

We believe that it is therefore essential that

esophogoscopy with mucosal biopsy be performed on all patients with radiographic or historical evidence of esophagitis and that the barium swallow alone not be relied upon to make a diagnosis of *Candida* esophagitis.

Candida septicemia probably arises from the esophagus in most instances. Sepsis is difficult to diagnose because despite dissemination to liver, spleen, kidneys, lungs, and occasionally retina, most blood cultures are negative [3, 22, 23]. When a blood culture is positive for *Candida* spp in this patient population, follow-up indicates that dissemination has usually occurred and treatment with amphotericin B is indicated even if the patient does not appear critically ill [23].

Urinary tract infection. These are relatively uncommon unless a distinct and specific predisposing factor exists, *e.g.* catheter or other form of instrumentation, obstructive conditions such as prostatic hypertrophy or prostatic infiltration with leukemic cells, or a past history of recurrent urinary tract infections. The etiologic agent seems related to the stool flora; *E. coli* infections develop early during hospitalization, *P. aeruginosa* or *Klebsiella* spp occur later. *Candida* spp or *Torulopsis glabrata* tend to occur only in patients with marked alterations in their microbial flora and (unless secondary to septicemia) are usually catheter-related. Strict aherence to a policy that catheters may only be used for a patient with shock or urinary obstruction has resulted in a marked reduction of urinary infections at the Baltimore Cancer Research Center (BCRC).

As with other sites, the minimal inflammatory response limits dysuria and pyuria. Diagnosis depends on examination of the urine for organisms (by the physician) and quantitative cultures. Differentiation of pyelonephritis from cystitis is difficult in the absence of rarely-detected costovertebral tenderness. Septicemia from urinary tract infection is relatively uncommon unless there is either significant obstruction or an indwelling catheter in association with granulocytopenia.

Bacteremia. Bacteremia is always secondary to infection somewhere. It is essential that the primary site of infection be detected so that appropriate local measures may be applied. Nearly one third of all infections in our patients with adult acute leukemia have an associated bacteremia, [4] although improved preventive measures (reduced organism acquisition, microbial suppression, and very prompt use of empiric antibiotics) in the past few years have markedly reduced the frequency of bacteremias [2, 24]. Pneumonia, anal lesions, and pharyngitis have been the primary sources. Previously urinary catheters and indwelling venous catheters (both now banned) were associated with frequent bacteremias. *Pseudomonas aeruginosa*, other Gram-negative bacilli, *Staphylococcus aureus*, and occasionally *Candida albicans* account for most septicemias. The latter organisms and *Torulopsis glabrata* tend to occur in terminal patients; *Candida* spp arise from the esophagus and intestine and *Torulopsis glabrata* originates in pneumonitis [25]. One must not be misled by a rather benign appearing lesion in the pharynx, in the anus, or a minimal infiltrate in the lungs, as these can be, and usually are major sources for bacteremias in these patients.

Hepatitis. Hepatitis is a serious problem among any patient population subjected to frequent blood product transfusions. At this institution more than 80% of adults with acute nonlymphocytic leukemia will develop hepatitis within a few months after their initial admission [26]. Not only is hepatitis frequent but often remains chronically active. The incidence of hepatitis at the BCRC has remained high despite screening of blood and blood products including platelets for the hepatitis-associated antigen. There is some epidemiologic evidence to suggest that this is parenterally-acquired hepatitis A or C rather than hepatitis B because of the early onset after admission and non-detection of the hepatitis antigen and antibody even with improved sensitive diagnostic techniques. An occurrence of hepatitis A has developed among bone marrow transplant patients [27]. These patients would greatly benefit if an effective vaccine were available [28]; however, before double blind clinical trials of the hepatitis B vaccine are initiated, the most common agent (A, B, or C) must be established.

Multiple myeloma

Infections associated with multiple myeloma have changed considerably in the past decade [29]. Previously, reduced serum opsonizing antibodies predisposed to pneumococcal and *Hemophilus* infections. Although these infections still occur, there has been a steady increase in the incidence of Gram-negative bacillary and *Staphylococcus aureus* infections. Modern intensive therapy of myeloma usually reduced the abnormal proteins with return, in time, of adequate functional serum antibodies so that the patient in remission has few infections. However, if the myeloma is either poorly responsive or rapidly recurrent, the disease, its therapy, and consequent myelo-suppression predispose to opportunistic infections similar to those occurring in patients with adult acute leukemia.

Lymphona

The patient with lymphoma who is recently diagnosed, receiving initial therapy or in remission tends to have relatively few infections whereas the patient who has recurrent or progressive disease develops infections which are quite similar to those found in adult acute leukemia and also those related to immunologic deficiences [3, 9, 30, 31].

Early lymphoma. The only common infection is *Herpes zoster* which occurs primarily following recent radiation therapy. Additional important predisposing factors to *Herpes zoster* are adjuvant chemotherapy following radiation therapy, female sex, and age less than 30 years.

There is also some evidence to suggest that patients who do not respond to a dinitrochlorobenzene skin test challenge or a relatively low lymphocyte count have a higher incidence of *Herpes zoster*. Preliminary data suggests that patients with noncaseating epitheloid granulomas have a reduced risk of developing zoster [15, 30, 32].

It should be remembered that *Herpes zoster* is contagious among predisposed patients with an incubation period ranging from 15 to 30 days; hence the high risk patient (i.e., the female treated with radiation and chemotherapy) should avoid exposure to those with either zoster or varicella [15]. Although often not a very severe disease, zoster most frequently occurs just when therapy has been completed, the patient is feeling physically and emotionally better, and both patient and physician are hoping for long term remission or cure. Fortunately, dissemination occurs rarely in "early" lymphoma [30].

Progressive lymphoma. The spectrum and frequency of infection is quite different for the patient with either initially unresponsive or recurrent lymphoma [30]. *Herpes zoster* is also common now but is more likely to disseminate or cause major complications such as encephalitis or pneumonia. Preliminary clinical observations suggest that *Herpes zoster* occurring more than a year after the completion of therapy often represents the recurrence of lymphoma. *In vitro* measures of cell-mediated immunity to the *Varicella-zoster* virus have shown impaired responsiveness before and during initial therapy and during recurrence but a return to normal within a year after the patient obtains a remission [33].

In addition to *Herpes zoster* there are a variety of other infections which tend to occur in patients with lymphoma but which tend to occur infrequently in acute leukemia. These include disseminated *Cryptococcus neoformans* infection, *Listeria monocytogenes* bacteremia, *Nocardia asteroides* infection, disseminated *Cytomegalovirus* infection, *Pneumocystis* pneumonia, and occasionally toxoplasmosis. In addition, the patient with a past history of tuberculosis may have a recurrence with rapid progression to disseminated disease. These are all infections which tend to occur in the patient with late stages of lymphoma (although any may occur earlier on less frequent occasions) and appear to be related to immunologic dysfunction and not just myelosuppressive therapy [3, 9, 30, 31].

However, patients with progressive or recurrent lymphoma who become granulocytopenic from either disease or its therapy also develop infections generally similar in frequency, severity, and etiology to those infections found in adult acute leukemia. Pneumonia is particularly common especially in the patient who has any form of pulmonary lymphoma. Rectal abscesses are uncommon but pharyngitis and esophagitis certainly occur. Tumor masses may cause obstruction which lead to infection in sites such as the urinary tract, the biliary tract or the middle ear. In these situations, as in acute leukemia, the etiologic agents are most frequently *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus* plus transfusion-associated hepatitis [30, 31].

Despite considerable published concern, there is no good evidence to suggest that splenectomy *per se* significantly increases the incidence or severity of infection (including pneumococcal or *Hemophilus* spp) in adults with Hodgkin's Disease [30]. However, it has been shown that months to many years later a very small percentage of normal individuals splenectomized for trauma may develop a serious pneumococcal infection thus suggesting that this possibility be considered when evaluating a patient with signs of sepsis [34].

Patients with Hodgkin's or non-Hodgkin's lymphoma follow the same patterns with regard to infection; however, since more patients with non-Hodgkin's lymphoma have either poorly responsive or rapidly recurring tumor, the oncologist is more likely to see cryptococcosis, pneumocystosis, toxoplasmosis, or *Cytomegalovirus* infections in these patients than in patients with Hodgkin's Disease who usually enjoy an extended complete remission.

The patient with advanced mycosis fungoides, a specific subtype of non-Hodgkin's lymphoma, has a very high tendency toward infections related to his skin lesions; these are most frequently caused by

Staphylococcus aureus. S. aureus bacteremia is their most common cause of death [35].

Solid tumors

Although there are many different types of solid tumors, there are certain general principles that underlie the etiology and presentation of infection in these patients as a group. First, obstruction leads to infection. Tumors obstructing ureter or urethra lead to urinary tract infection, tumors obstructing the biliary tree lead to ascending cholangitis or occasionally hepatic abscess, tumors obstructing a bronchus lead to pneumonia, and tumors in and about the sinuses or mastoid region may predispose to sinusitis, otitis or even meningitis. Second, local lesions may predispose to local areas of infection, *e.g.* an ulcerating breast carcinoma may be associated with a local cellulitis. Although their rarity in acute leukemia is notable, anaerobic infections are common in patients with solid tumors, especially in association with obstructive lesions. Finally, iatrogenic maneuvers such as placement of urinary or venous catheters frequently predispose to urinary tract infection or bacteremia. In each of these situations, therapy which causes granulocytopenia or immunologic impairment may exacerbate an otherwise localized infection.

Central nervous system tumors

These patients must be separated from others because the types of infection which develop are directly related to their neurologic impairment. Included here are patients with primary tumors, metastatic carcinomas, or meningeal leukemia. The urinary tract is probably the most common site of infection because of either indwelling urinary catheters or neurologic impairment with micturition. The second most common infection is an aspiration pneumonia related to decreased mentation and/or loss of gag reflex. Although uncommon, it is of interest that patients with cancer other then lymphoma who develop *Herpes zoster* tend to have either a primary central nervous system tumor or a metastasis involving either the brain or the spinal cord for which the patient has had radiation therapy.

Clinical settings

When initially evaluating a possible infection, the determination of existing potential factors will establish the likely type of infection.

Patients with marked granulocytopenia. Patients with the following diagnoses can be grouped together: adult acute leukemia, chronic myelocytic leukemia in blastic crisis, advanced multiple myeloma, and recurrent or progressive lymphoma [3, 36]. In these settings, if the patient is receiving some form of antitumor therapy producing granulocytopenia, the chance that the patient has a serious infection is considerably greater than the possibility of drug-related or tumor-related fever. However, it is pertinent to determine whether the patient had recently received any drugs or blood product which might have caused fever. In addition, contaminated blood, blood products, or i.v. infusions may represent a source for transient or persistent infusion of bacteria or yeasts. Intravenous fluid infusing for more than 24 hr, i.v. tubing not changed within the last 24 hr, and butterfly needles in place for more than 48 hr are other conditions to check immediately. If an i.v. polyethylene catheter is in place, it should be considered as a highly potential source of bacteremia. Urinary catheters (which, like venous catheters can rarely be justified in these patients) are prime suspects for infection causation and if at all possible should be removed.

The history must be meticulously complete with emphasis upon subtle or seemly minor changes, e.g. minimal cough, slight dyspnea, change in bowel movements or pain with defecation, sore throat, and substernal pain or dysphasia. The history should include questions which may help in the early suspicion of hepatitis (fever frequently preceeds jaundice). Examination must likewise be exceedingly thorough and must include a careful inspection of the anal margin and a careful digital examination ofthe rectum. Minimal lesions must not be ignored because the usual manifestations of inflammation simply do not develop appropriately in this group of patients [3, 4, 36, 37]. The physician should obtain a urine specimen and personally examine it for evidence of bacteria. Granulocytopenia usually precludes finding urinary leukocytes.

Initial cultures should include, at a minimum, two sets of blood cultures (drawn from two separate venipunctures), plus cultures of the throat, nose, and rectum and any other site which looks even minimally suspicious. Minor areas of cellulitis frequently require the injection of a small amount of saline (without preservative) with aspiration to obtain material for culture. A blood sample should be sent for hepatitis antigen assay and the patient should have posterior–anterior and lateral chest X-rays.

If all the above are negative but the patient has had onset of new fever and appears even minimally toxic, then empiric broad spectrum antibiotics should be immediately administered. Prolonged antibiotic administration clearly predisposes to further infection, especially with fungi; therefore the

physician should be prepared to discontinue the use of these antibiotics within a few days if no evidence for infection is ultimately found [24, 38, 39]. The limulus assay for endotoxin may possibly prove to be helpful when infection is suspected but cannot be proven [40].

Alternatively, if the examination and history are negative but blood cultures return positive, the physician must continue searching for the site of origin of the bacteremia. This is essential because appropriate therapy often depends not only upon antibiotics but also removal of catheters, local therapy to an abscess, relief of obstruction caused by tumor, etc. It is therefore important that chest X-rays and a complete history and physical examination including anorectal evaluation be repeated daily. It is not at all infrequent for the site of an infection not to be detected for two to three days after the initial manifestations of fever and/or toxicity.

There is an increasing incidence of fungal infections including candidiasis, aspergillosis, and mucor mycosis following multiple past infections, prolonged periods of blood spectrum antibiotics and persistent granulocytopenia [3, 9]. A careful history to detect esophagitis followed by barium swallow and esophagoscopy with biopsy is often very appropriate in these patients. All too often, the diagnosis of disseminated candidiasis is made only at postmortem examination when it should at least be suspected from a suggestive history plus findings of high fever, shaking chills, yeasts in the urine, and yeasts in high number in the throat and stool cultures. Usually, blood cultures are negative but even a single positive blood culture which grows Candida must be construed as evidence of disseminated candidiasis in these patients [23].

Because of the high frequency of fungal pneumonias, it is essential that the etiologic agent of even a minimal pulmonary infiltrate be defined as promptly as possible [4, 12, 41]. Over the years at this institution it has become exceedingly clear that sputum examination is nearly worthless because of a combination of poor sputum production and contamination by posterior pharyngeal flora [4, 12, 13]. Transtracheal aspiration is therefore performed at the first evidence of pneumonia, preferably before antibiotic therapy is begun. Unfortunately, since both history, examination and chest X-ray are frequently negative during the first day or two of a pneumonia the transtracheal aspiration is usually not performed until after antibiotics have been in use for a day or two. Although transtracheal aspiration is far superior to expectorated sputum for diagnosis, many infections cannot be diagnosed unless material can be scraped from small bronchioles or removed directly from alveoli or interstitium. This requires either a percutaneous or open lung biopsy or the use of bronchial biopsy or brushing [13]. At BCRC, Aisner *et al.* have utilized transcricoid selective bronchiole brushing for the early diagnosis of pneumonia in these patients. A small directable catheter is placed across the cricoid membrane, bypassing oropharyngeal contamination, and is fluoroscopically directed to the infiltrate. Tiny brushes are passed via the catheter into the lesion, withdrawn and evaluated with histologic, cytologic and cultural techniques [13].

Eight of 11 consecutive pulmonary infiltrates, whose etiology was unknown after smear cultures of sputum and transtracheal aspiration, had the diagnosis proven by the brushing technique. Included were three cases of *Aspergillus* pneumonia, two *Torulopsis glabrata* pneumonias and one each of *Staphylococcus aureus, Peptostreptococcus* sp pneumonia. Knowledge of the etiologic agent allowed the institution of sufficiently prompt appropriate, specific single agent therapy that survival has improved. We believe that the addition of the brushing technique to the orderly evaluation of presumed infectious pulmonary infiltrates, prior to instituting antimicrobial therapy, should increase accurate diagnosis and thus lead to more appropriate therapy in this patient population [13].

A further discussion of the differential diagnosis of candidiasis, mucor mycosis, aspergillosis, and cryptococcal infections can be found in the excellent short article by Bennet [42].

Lymphoma (early). Infections are uncommon in the patient with Hodgkin's disease or non-Hodgkin's lymphoma both initially and during therapy unless there has been urinary catheterization or obstruction of bronchiole, biliary tree, or urinary tract by tumor. *Herpes zoster* is the one common infection [15, 30].

Lymphoma (unresponsive or recurrent). The nature of infectious complications is markedly different for the patient with recurrent or unresponsive lymphoma plus myelosuppression and/or immunosuppression (from disease or therapy). These patients tend to become infected with the same organisms in many of the same sites as the patients with adult acute leukemia. In addition, they are highly prone to *Listeria monocytogenes* bacteremia, disseminated tuberculosis, salmonellosis, *Cryptococcus neoformans* meningitis, *Cytomegalovirus* infection, and toxoplasmosis. It is therefore imperative that not only should the procedures for acute leukemia be meticulous observed but that the patient be carefully considered for the infections apparently associated

with immunologic dysfunction. Pulmonary infiltrates, with or without fever or headache should suggest cryptococcal infection. It is not uncommon for the pulmonary infiltrate to regress while meningoencephalitis develops. Diagnosis is best made with cultures of spinal fluid, sputum, blood, and urine. Spinal fluid and serum assays for cryptococcal antigen are helpful if cultures are negative but the diagnosis is strongly suspected. *Listeria* infections usually originate in the lungs; the return of a blood, spinal fluid, or pleural fluid "contaminated with diphtheroids" should prompt a laboratory review for *Listeria monocytogenes.*

Pneumocystis carinii is a common cause of rapidly progressive bilateral (usually) interstitial pneumonia. In children with acute lymphocytic leukemia, *Pneumocystis* usually develops when methotrexate and prednisone are withdrawn during remission. In adults, *Pneumocystis* pneumonia tends to occur in patients on intensive immunosuppressive therapy for progressive lymphoma and can be diagnosed with the brushing technique or lung biopsy. Other causes of pneumonitis in this type of patient include those caused by *Toxoplasma gondii* and *Cytomegalovirus* in addition to the organisms frequent in adult acute leukemia.

Although it is true that the patient with cancer is often more susceptible to opportunistic infections than is the average individual, it is important to remember that each patient must be looked at as an individual and that the specific tumor, the status of that tumor, the type of therapy the patient is receiving, iatrogenic procedures, previous antibiotic therapy, immunologic dysfunctions and other factors all are important in predisposing to each of the types of infections discussed above. In attempting to diagnose the cause and site of an infection in a patient with cancer it is essential to first look for and understand the various predisposing factors as these will be the major clues towards indicating the most likely type or types of infection which might be occurring in that individual patient. Second, utilizing the information already obtained, the patient must be examined in minute detail including those specialized examinations ranging from anorectal examination to transtracheal bronchiole brushing. Third, recognize that the usual manifestations of inflammation are often either absent or markedly altered by the disease or its therapy such that minor alterations from normal may be representative of serious infection.

If the physician will but take the time to understand the specific setting, utilize those clues available, and to be both meticulously thorough and willing to repeat examinations as necessary, then the majority of infections will be both promptly and accurately diagnosed.

*Table 1. Infections in patients with cancer: incidence, site, organisms**

Adult acute leukemia	Pneumonia	*Pseudomonas aeruginosa*
	Anorectal lesions	*Escherichia coli*
	Pharyngitis	*Klebsiella pneumoniae*
	Urinary infections	*Staphylococcus aureus*
	Skin lesions	Hepatitis viruses
	Hepatitis	*Candida albicans*
	Esophagitis	*Aspergillus*
Lymphoma ("early", during initial therapy)	Herpes zoster (localized)	*Varicella-zoster* virus
Lymphoma (progressive or recurrent disease)	See acute leukemia	See acute leukemia
	Herpes zoster (local or disseminated)	*Varicella-zoster* virus
	Meningoencephalitis	*Cryptococcus neoformans*
		Pneumocystis carinii
		Listeria monocytogenes
		Toxoplasma gondii
		Cytomegalovirus
Solid tumors	Pneumonia	*Escherichia coli*
	Urinary infection	*Staphylococcus aureus*
	Abscess	Anaerobic organisms
Brain tumors	Pneumonia	Mouth flora
	Urinary tract	*Escherichia coli*

* Sites and organisms noted in approximate descending order of frequency. Organisms do not necessarily correlate with adjacent-listed site.

Table 2. Infections in cancer patients: predisposing factors

Adult acute leukemia	Granulocytopenia Leukocyte dysfunction Mucosal alterations Iatrogenic procedures: urinary catheters, venous catheters, contaminated infusions or blood products Hospital acquisition of organisms Overcrowding, poor staff hygiene–contaminated food, water, medications
Lymphoma (recurrent or progressive)	Granulocytopenia Immunologic dysfunction Obstruction of natural passages Iatrogenic procedures
Solid tumors	Obstruction of natural passages Iatrogenic procedures Granulocytopenia (chemotherapy)
Brain tumors	Neurologic dysfunction

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GRAM-NEGATIVE ROD BACTEREMIA IN CANCER PATIENTS. A REVIEW WITH EMPHASIS ON THE ANTIBODY RESPONSE*

Stephen H. Zinner†, and William R. McCabe‡

†Division of Infectious Diseases, Section of Medicine, Roger Williams General Hospital, Brown University, Providence, RI, U.S.A.

‡Department of Infectious Diseases, Boston University Medical Center, Boston, MA, U.S.A.

The nature and severity of Gram-negative rod bacteremia in patients with cancer has been examined. Patients with low levels of circulating neutrophils, low levels of antibody at the time of onset of sepsis and patients infected with antibiotic resistant strains are at particular risk of dying from this infection. Overall, infection remains the most important cause of death in patients with hematologic malignancies and solid tumors. Control efforts should be directed at developing more effective methods of maximizing efficacy of currently available antimicrobial agents and the search for new potent and safe antibiotics must continue. Moreover, there is a real and pressing need to prevent these infections in this susceptible population and efforts should continue in the direction of optimal isolation and protection of the patient at risk as well as exhaustive studies of the mechanisms of immunity to Gram-negative rods and their potential enhancement in cancer patients.

INTRODUCTION

WITH the introduction of antimicrobial drugs effective against a broad spectrum of microorganisms, the past three decades have seen a remarkable change in the nature and practice of infectious diseases. The patient with malignant disease, of course, has always been at particular risk of infection, and the development of potent anti-tumor agents has increased that risk as an unwanted side effect of this therapy. The purpose of this review is to highlight the role of bacteremia due to Gram-negative rods in patients with cancer with a particular emphasis on the immune response to these organisms.

Although the Enterobacteriaceae and Pseudomonadaceae have been known to medical microbiology for many decades, it is only within the past 25 years that these organisms have achieved clinical prominence. One review in 1965 [1] referred to Gram-negative rod bacteremia as "The New Disease." The studies of Finland [2] at the Boston City Hospital have documented clearly the nature and extent of the changing ecology of bacterial infections associated with the development and frequent use of antimicrobial agents. The introduction and popular use of the sulfonamides and penicillin led initially to a striking decrease in the occurrence and mortality of bacteremia due to *Diplococcus pneumoniae* and Group A beta hemolytic streptococci. This decline was associated with a rise in the number of patients and mortality due to bacteremia with *Staphylococcus aureus* and Gram-negative rods. The increase in bacteremia due to Gram-negative bacilli continued despite, and perhaps related to, the use of antibiotics effective against these organisms. As new antibiotics effective against this class of organisms have been developed, there have been changes in the patterns of antibiotic susceptibility [3–6]. The discovery of episomal transfer of resistance to several antibiotics in Gram-negative bacilli has helped to explain some of the selective pressures responsible for these changes [3, 7, 8].

The increasing frequency and mortality associated with bacteremia due to Gram-negative rods have been documented also by McCabe and Jackson [9, 10] in their review covering an 8-year period from 1951 to 1958. Similarly Freid and Vosti [11] reported a rise in yearly incidence of these infections from 0·7 per 1000 admissions in 1959 (the year the Palo Alto-Stanford Hospital Center opened) to 2·5 cases per 1000 admissions in 1965–1966.

Although it is clear that patients with cancer are not the only group of individuals infected with Gram-negative rods, virtually every series reviewed included a substantial proportion of cancer patients. The early review by McCabe and Jackson [9, 10] as

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well as several subsequent studies [11–14] emphasized the importance of considering the underlying disease state of the patient in evaluating incidence, severity and outcome of bacteremia due to Gram-negative rods. The original classification of McCabe and Jackson [9] was based on the severity of the underlying disease rather than the presence of the disease itself. They studied patients with "rapidly fatal" underlying disease (which included acute leukemia or blastic crises in chronic leukemia) who were not expected to survive for 1 year, patients with "ultimately fatal" underlying disease (which included chronic leukemia, lymphoma, metastatic carcinoma, polyarteritis, advanced stages of chronic hepatic or renal disease, etc.) who were not expected to survive more than 4 years, and patients with "nonfatal" underlying disease (*e.g.* diabetes, genitourinary tract, gastrointestinal or obstetrical diseases) who were expected to survive for at least this duration. In subsequent studies [15], and for the sake of convenience in the remainder of this paper, the rapidly fatal and ultimately fatal disease categories have been combined into one group (ultimately fatal disease, UFD) and were compared to the nonfatal disease group (NFD). It should be stressed that not all of the data reviewed here deal exclusively with cancer patients and those studies which do so will be indicated clearly.

Several observations made in the early studies deserve consideration. First of all, host factors appear to be the most important determinants of the outcome of bacteremia. Fatality rates for UFD patients range from 65 to 73 % or higher compared to 11–16 % in patients with NFD [10–13]. Increased mortality in general has been related also to aging, occurrence of shock, presence of azotemia and acidosis, and in some studies the development of infection in the hospital [10–14].

The infecting organisms

Although almost every clinically important Gram-negative rod has been identified in patients with bacteremia, the most frequently identified organisms in patients with malignant disease include *Escherichia coli,* Klebsiella species, *Pseudomonas aeruginosa,* Proteus species, Salmonella, Enterobacter species, *Serratia marcescens* and rarely Providencia species, *Mima polymorpha, Moraxella osloensis* and *Herellea vaginicola,* now referred to as varieties of *Acinetobacter calcoaceticus.* A review of the Gram-negative bacteremias from 1964-1969 at the Memorial Hospital for Cancer and Allied Diseases in New York revealed *E. coli,* Klebsiella–Enterobacter species and *Pseudomonas aeruginosa* to be the most common infecting organisms [16]. Similar data have been obtained at other institutions [3, 9, 11–14, 17–19].

Most large series do not support statistically any enhanced virulence for a particular organism. Bryant *et al.* [12] did report significantly increased mortality in patients with *Pseudomonas aeruginosa* bacteremia compared to other Gram-negative rods. However, this statistic may reflect the fact that the distribution of patients with Pseudomonas bacteremia was more heavily weighted with RFD patients than was bacteremia with the other organisms studied. In addition, in the Memorial Hospital series, Pseudomonas was responsible for 84 % of fatal bacteremia in patients with acute monocytic or myelogenous leukemia [16].

Recent experience with bacteremia due to *P. aeruginosa* has been reviewed by Tapper and Armstrong [20]. Although the rates of isolation of Pseudomonas from blood cultures of patients in this cancer hospital declined from 0·82 % of 6463 cultures in 1968 to 0·27 % of 7276 cultures in 1972, an overall mortality rate of 69 % was recorded in 1972. Despite the widespread use of carbenicillin and gentamicin in this institution, this combination was more effective than gentamicin alone in neutropenic cancer patients. The minimum inhibitory concentrations of these antibiotics were lower for Pseudomonads isolated from survivors than for those from the fatal cases. Young and Armstrong have demonstrated that most strains of Pseudomonas isolated from patients in a cancer center resist the bactericidal activity of 10 % pooled normal serum [21]. This finding may, in part, explain the particularly high mortality rate in the UFD patient infected with this organism. Enterobacteriaceae isolated from patients with bacteremia also resist serum bactericidal activity [22, 23]. Umsawasdi *et al.* [24] reported a rise in the frequency of Klebsiella bacteremia associated with decreased fatality from Pseudomonas bacteremia attributed to successful therapy with carbenicillin.

Polymicrobial bacteremia due to several species of Gram-negative bacilli may also occur in cancer patients. Patients with malignancies accounted for 22 % of 46 patients with more than one organism in blood cultures from the Mayo Clinic over an 18-month period [25]. An earlier survey from the National Institutes of Health which included a majority of patients with malignancies reported two organisms from 8 % of patients with positive blood cultures, not all of which were Gram-negative rods [26].

Recent years have seen an increase in interest in the Bacteroides and other anaerobic Gram-negative bacilli due to improved and more readily available

bacteriological techniques [27–29]. Patients with cancer have their fair share of infections with these organisms. Kagnoff *et al.* [30] reviewed their experience with 55 cancer patients with Bacteroides bacteremia and found an overall mortality rate of 35 %. Bacteroides bacteremia was more common in patients with solid tumors, especially gastrointestinal or genitourinary, than in patients with leukemia or lymphoma. They stressed the finding that the majority of their isolates required from 3 to 8 days' incubation before the Bacteroides were identified. Transient bacteremia was noted in four patients. These organisms are not usually very pathogenic and comprise almost 98–99 % of the stool flora. Thus, patients with abdominal or pelvic tumors are at enhanced risk for infection with the anerobic Gram-negative rods.

Similarly, Bodner *et al.* [31] emphasized the frequency of malignancy and other debilitating diseases in their 39 patients with Bacteroides bacteremia. Neoplasms, especially colon or uterine cancers, were present in 57 (23 %) of 250 patients with Bacteroides bacteremia reviewed by Felner and Dowell [32], who reported a 54 % mortality rate. Finegold *et al.* [33] reviewed several studies and reported 43 % of their patients with anaerobic infection had underlying malignancy.

Clinical presentation of Gram-negative rod bacteremia

The clinical presentation of Gram-negative bacteremia is reasonably familiar to today's physician. In most series the sudden onset of striking fever, usually 102–104°F, is present in almost all patients [1, 10, 14]. Fever is most prominent on the 1st day of the illness. Chills are present in about 50 % and were inversely proportional to the severity of the underlying disease state [10]. Shock (or the development of blood pressure less than 90/60 mm Hg or a fall of 70 mm Hg of systolic pressure) is found in 40–50 % of patients in most series [10–14] and is associated with a significant increase in mortality regardless of therapy. Nausea, vomiting and diarrhea may be present in up to 1/2 of patients with Gram-negative rod bacteremia. Tachycardia, confusion or stupor, oliguria (even in the absence of shock) and hyperpnea (often with respiratory alkalosis developing without apparent cause [34]) may also be presenting signs. A few patients may be hypothermic. Ecthyma gangrenosa, widely scattered necrotic skin lesions, are typical of bacteremia with *Pseudomonas aeruginosa,* but may be seen also with some Aerobacter and Aeromonas species [35].

The white blood count may be elevated, but usually in immunosuppressed patients with malignant disease it is depressed. In most studies, increasing mortality is associated with increasing degrees of neutropenia. Patients may be anemic or thrombocytopenic, and abnormalities of the clotting system with or without frank disseminated intravascular coagulation may occur [35]. Acidosis and azotemia may be present and are associated with an increased mortality rate in elderly patients [12, 14]. There may also be changes in the ECG and elevations in serum uric acid, bilirubin and other liver function tests.

Predisposing features and sites of infection

There are many features of the patient with cancer or leukemia with or without the effects of chemotherapy which predispose to infection in general. As reviewed recently by Armstrong *et al.* [16], the host with neoplastic disease may have altered resistance secondary to: (1) decreased numbers and activity of polymorphonuclear leukocytes (as in acute leukemias); (2) defective cell mediated immunity (as in Hodgkin's disease, lymphoma and some leukemias); or (3) altered serum antibody status (as in chronic lymphatic leukemia, myeloma, etc.). All forms of immunity may be altered by presently employed therapeutic modalities. In many series, occurrence and survival of Gram-negative rod bacteremia is related directly to the number of circulating leukocytes, especially neutrophils [2, 18, 20, 36]. The role of white cell transfusion in the infected cancer patient has been referred to elsewhere in this symposium. Similarly, and not studied as extensively, serum levels of immunoglobulins (IgG and IgM) may be related directly to survival as reported for Pseudomonas bacteremia by Tapper and Armstrong [20].

In the series reviewed, treatment with radiation or chemotherapy had an adverse effect on survival from Gram-negative rod bacteremia [18, 20]. Surgical procedures, tracheostomy, indwelling plastic intravenous catheters, contaminated respiratory therapy apparatus, dialysis catheters, etc. are common modalities which may predispose patients to Gram-negative rod bacteremia by bypassing the usual host defense mechanisms [18, 35, 37, 38].

The roles of corticosteroids and antibiotics as predisposing features deserve special attention. In two series of infection in cancer patients, pre-sepsis therapy with corticosteroids had a definite adverse effect on survival [18, 20]. Similarly, the mortality rates were considerably higher in patients who had received antibiotics than in patients not so treated. Presumably, this reflects selection of more resistant strains in the antibiotic-treated patients.

Schimpff *et al.* [39] have utilized extensive environmental and patient surveillance cultures to

attempt to define the relation of presepsis colonization to eventual bacteremia. In several studies at the Baltimore Cancer Research Center, bacteremia was most frequently due to *P. aeruginosa,* Klebsiella species and *Escherichia coli.* The overwhelming majority of these bacteremias were preceded by colonization with the same organisms of the patient's nose, gingival surface, rectum, axilla, groin, perineum, vagina, etc. Almost 50 % of the organisms responsible for the bacteremias were acquired in the hospital. The prevention of Gram-negative rod infections in cancer patients has been discussed elsewhere in this symposium.

The usual sites of infection vary with the hospital population. In most general hospitals the urinary tract is the most frequent source of Gram-negative rod bacteremia, especially in the presence of catheterization or instrumentation of this system [10, 19]. However, two studies [20, 39] have commented on the relative infrequency of urinary infections as a cause of Gram-negative rod bacteremia in patients with acute leukemia. Other frequently implicated sites of infection include the lungs, gastrointestinal tract, especially the rectum and perirectal area, and the skin (including wounds and i.v. catheters).

The antibody response in Gram-negative rod bacteremia

The mechanisms involved in the host response to Gram-negative rods are incompletely known relative to our knowledge of the response to Gram-positive cocci. Certainly, phagocytosis by white blood cells, immunoglobulins, antibody of the opsonizing type, the complement system and the serum bactericidal system must be involved. The relative roles of these systems in protection against Gram-negative rod infection as well as protection against the lethal complications of infection remain to be elucidated fully.

The complex antigenic structure of the Gram-negative bacilli has received considerable attention in recent literature. The major antigens of the cell wall include the somatic O antigen; the K or capsular surface antigen; the L, A and B antigens of *E. coli* and the flagellar H antigen [40]. Recent interest in the O antigen or lipopolysaccharide (LPS) of the cell wall has revealed this to consist of three regions [41]: Region I contains the O specific polysaccharides which impart serological specificity to the organism; Region II is the core polysaccharide which is common in composition to all Enterobacteria, but differs for various species in the linkage and location of the components; Region III consists of Lipid A and is linked to the core polysaccharide by 2-keto-deoxyoctonate trisaccharide (KDO).

The development of rough (R) mutants of Enterobacteria which were deficient in enzymes responsible for linkage or production of various components of LPS has made possible studies of the immunogenicity of these particular components. A rough mutant (R_e) of *Salmonella minnesota* has been prepared and its cell wall consists only of Lipid A-KDO [41]. Presumably this component, expressed by the R_e mutant, is shared by all species of Enterobacteriaceae and perhaps by Pseudomonadaceae.

One of the paradoxes in Gram-negative rod infections is that high titers of antibody may exist but not protect against subsequent infections. Although "natural antibody," presumably of the IgM class, does exist in normal serum [21, 42] as does opsonizing antibody (also presumably IgM) [21, 43], Vosti has demonstrated that neither natural antibody nor high titers of O specific antibody protected patients against Gram-negative rod infections of the urinary tract [44].

On the other hand, immunization of experimental animals has been shown to exert a protective effect against Gram-negative rod infection. Freter [45] prevented the lethal effects of cholera by immunizing with boiled *V. cholera* or vibrio derived mucinase. *E. coli* antiserum has been shown to protect mice from the lethal effects of i.p. endotoxin from the same organism [46]. Also, a protective effect of anti-serum against endotoxin has been reported to be independent of O antibody [47]. These and other studies raised the possibility that antibodies to an antigen shared in common by all enteric bacilli might have a protective role in Gram-negative rod infections.

A likely early candidate was the "common antigen" of Kunin [48]. Antisera to *E. coli* 0 14, cross reacts with all Enterobacteriaceae except Serratia. McCabe, however, has shown that antibodies to common antigen protected neither animals from the subsequent challenge with heterologous bacteria [49] nor humans with Gram-negative bacteremia [50]. A more feasible antigenic component whose antibodies were likely to possess protective activity was the Lipid A-KDO complex which is present in all Enterobacterial cell walls and represented by the R_e mutant. In the experimental animal, McCabe [51] has shown that active and passive immunization of mice with a R_e mutant of *Salmonella minnesota* afforded protection of mice against i.v. challenge with lethal infective doses of *Klebsiella pneumoniae* and *Proteus morganii.*

A subsequent clinical study [15] demonstrated that Gram-negative rod bacteremic patients who had high serum titers of R_e antibody present at the onset

of infection had a decreased frequency of shock and death due to their infection. Although the frequency distribution of R_e antibody titers was similar in patients with UFD and NFD, significant protection, as indicated by a decreasing frequency of shock and death with an increasing R_e antibody titer, was found in the UFD ($r = -0{\cdot}26$, $P < 0{\cdot}02$) and when UFD and NFD groups were combined ($r = -0{\cdot}175$. $P < 0{\cdot}05$).

In this clinical report, no protective effect (*i.e.* decreased frequency of shock or death) was found for increasing hemagglutinating antibody (HA) titer to the homologous O antigen of the patient's infecting organism. Since the HA test measures preferentially IgM or 19S antibody, to further evaluate the protective role of antibody in patients with Gram-negative rod bacteremia specific IgG antibody titers directed against the infecting organism were measured in 188 patients at the Boston University Medical Center. Patients were classified into two categories according to their underlying disease: UFD (103 patients) and NFD (85 patients) as above. The indirect immunofluorescent antibody technique (IFA) was used to measure IgG and IgM antibody. The fluorescent antisera used were tested for monospecificity by immunodiffusion and with the IFA test after absorption with homologous immunoglobulin. Hemagglutinating antibodies also were studied in these sera with and without 2-mercaptoethanol reduction. The frequency of shock and death was determined in each patient group according to the antibody titer, and this relation is expressed as the point biserial correlation coefficient.

As expected from other reported studies, the frequency of shock, death and both events were significantly related to the underlying disease (Table 1). Although shock was somewhat more frequent in UFD patients infected with Proteus sp., Providencia and Pseudomonas than with other organisms, there was no significant difference in mortality rates with the various organisms isolated (Table 2).

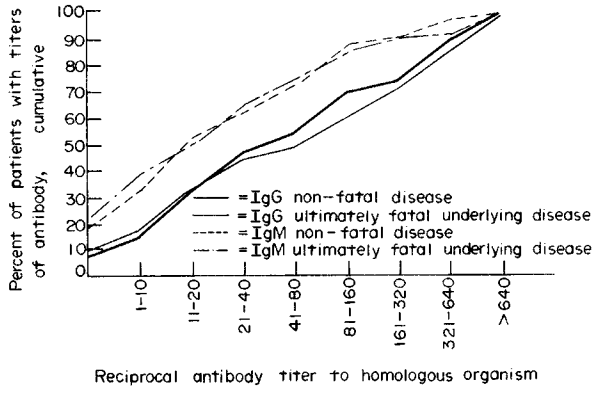

Fig. 1. Cumulative IgG and IgM antibody titer directed against the infecting organism in 188 episodes of Gram-negative rod bacteremia according to underlying disease.

Table 1. Frequency of complicating events by underlying disease

Disease group	*N*	Shock	Death	Shock and death
Non-fatal	85	21 (25 %)	7 (8 %)	6 (7 %)
Ultimately fatal	103	43 (42 %)	24 (23 %)	21 (20 %)
Total	188	$\chi^2 = 6{\cdot}02$	$\chi^2 = 7{\cdot}68$	$\chi^2 = 6{\cdot}73$
		$P < 0{\cdot}025$	$P < 0{\cdot}01$	$P < 0{\cdot}01$

Table 2. Distribution of complicating events by infecting organism and underlying disease

Organism	Nonfatal underlying disease			Ultimately fatal underlying disease		
	N	Shock	Death	*N*	Shock	Death
E. coli	36	8 (22 %)	3 (8 %)	61	18 (30 %)	11 (18 %)
Klebsiella-Enterobacter-Serratia-	26	7 (27 %)	1 (4 %)	24	11 (46 %)	5 (21 %)
Proteus–Providencia	12	2 (17 %)	0	8	8 (100 %)*	4 (50 %)
Pseudomonas	8	3 (38 %)	2 (25 %)	8	7 (88 %)†	4 (50 %)

*Significantly different from *E. coli*, $P < 0{\cdot}05$.
†Significantly different from K-E-S, $P < 0{\cdot}05$.

As indicated in Fig. 1, the distribution of both IgG and IgM antibody titers did not differ significantly for the 2 patient categories; thus, the actual titers of these antibodies were similar for UFD and NFD patients. However, when both patient groups are considered together, IgG titers were highest in infections caused by *Escherichia coli* and were lowest in patients with Pseudomonas bacteremia.

As published previously [15], there was no correlation between hemagglutinating antibody to the homologous O antigen and frequency of complications of bacteremia. However, increasing titers of specific IgG antibody present at the onset of sepsis did correlate with a significant decrease in the frequency of shock or death in both patient groups ($r_{pbi} = -0{\cdot}29, P < 0{\cdot}01$). Overall, high titers of IgM were not associated with a decrease in these complications.

Moreover, when the previously studied anti R_e titers were examined according to the level of IgG antibody to the infecting organism, the protective effect of R_e antibody was independent of the protective effect of high titers of IgG antibody.

Although this study was not performed exclusively in patients with cancer, a large proportion of the UFD group had malignant diseases. These results suggest that IgG antibody to the O antigen does have a protective role in patients with bacteremia due to Gram-negative rods. Obviously, the role of antibody in the prevention of bacteremia cannot be evaluated in this study since all patients were infected when the sera was collected.

The detailed mechanisms by which antibodies exert this protective effect have not been elucidated. Young and Armstrong [21] have demonstrated opsonizing activity for Pseudomonas in normal sera and increased opsonizing titers were found in convalescent sera. They suggested that IgM may be the principal component of opsonizing activity in normal sera but that both IgG and IgM are involved in opsonic activity in convalescent sera. In an animal model system measuring uptake of bacteria by phagocytes, Smith *et al.* [52] reported IgG in hyperimmune sera to be a more effective opsonin than IgM. Bjornson and Michael [53] reported that IgG antibodies were required for opsonophagocytosis of *P. aeruginosa* and that immune IgG eliminated the need for properdin and C3 proactivator in the *in vitro* opsoninization of these organisms.

The patient with cancer or other immunodepressing condition, is at increased risk for developing Gram-negative rod bacteremia, and once infected, is more likely to succumb to the complications of the infection than is a normal host. The implication of the above studies is that it may become possible to develop an immunogenic stimulus shared by most Gram-negative bacilli, such as the cell wall components of the R_e mutant, which might be useful in enhancing resistance to Gram-negative rod infection in this most susceptible population. Since patients with neoplasms have been reported to mount an antibody response, however limited, to a Pseudomonas vaccine, the theoretical possibilities outlined above already have some clinical precedents. Since the outcome of therapy has changed so little in the past two decades, all efforts should be directed toward prevention of infection and if possible toward development of potential boosters to augment circulating immunoglobulin antibody.

Treatment of Gram-negative rod bacteremia

This subject has been reviewed recently in several publications and other reports in this symposium have dealt with this area [54–60]. In reviewing most studies, the selection of an antibiotic which is "appropriate" for the infecting organism seems to be the single most effective therapeutic modality [3, 10, 12–14, 18]. The adjunctive use of fluids, respiratory therapy, white cell transfusions, vasopressors and other supportive measures have also been reviewed elsewhere [10–14, 55].

The role of large pharmacologic doses of adrenal corticosteroids in the treatment of Gram-negative rod bacteremia remains unsolved. A recent critical paper [61] has summarized the adherence to standards for the evaluation of a therapeutic effect of steroids in infection. Weitzman and Berger reported that none of the studies of Gram-negative rod bacteremia met each of the standards of methodology necessary to resolve this controversy. However, in the one study that met all but one standard [62], Klastersky was unable to show any benefit for steroids in a double-blind study in serious Gram-negative rod infections in patients with cancer.

The appropriate antibiotics should be based on thorough sensitivity testing. Prior to the identification of the organism, most clinicians recommend the use of an aminoglycoside antibiotic such as gentamicin, amikacin, sisomicin, tobramycin, kanamycin (not active against Pseudomonas), etc. and either a cephalosporin (cephalothin, cefazolin, cephradine) or a "broad spectrum" penicillin as ampicillin, carbenicillin or ticarcillin. These drugs should be used in maximal doses, and it may be necessary to use doses even higher than usually recommended. Klastersky [63] has documented the importance of adequate antibacterial levels in determining the outcome of therapy of bacterial

infections. In this study of serious bacterial infections (about 2/3 were due to Gram-negative rods) in patients with cancer, the determination of antibacterial activity showed a significant correlation between therapy and peak serum antibacterial activity. The increased efficacy of synergistic combinations of antibiotics is well described in these patients [54, 64].

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GNOTOBIOTICS IN HEMATOLOGY: IMPROVEMENT OF TREATMENT OF ACUTE LEUKEMIA

Manfred Dietrich

Department of internal Medicine, Hematology Division, Center for Internal Medicine and Pediatrics, University of Ulm/Donau, Germany

Many patients with hematological disorders are at high risk of infection. Granulocytopenia, impairment of cellular immunity and lesions of protective tissues are the most important predisposing factors. Antibiotic therapy fails to control infection in a considerable proportion of these patients. Transfusion of compatible granulocytes may possibly increase the efficiency of antimicrobial therapy. Yet, mortality from infection remains high. Animal experiments have demonstrated that effective prevention of infection and reduced mortality can be achieved by antibiotic decontamination and use of germ-free techniques after grafting of incompatible bone marrow with subsequent graft-versus-host reaction. Similar techniques can be applied in medicine. Most experience in gnotobiotic care has occurred in relationship to patients with acute leukemia. The results of several working groups have shown that there is clear reduction of acquisition of new microbes, and mortality from infection, possibly longer remissions, longer overall survival, and higher remission rate. Yet, a number of problems remain, such as incomplete decontamination, possible development of resistant microbial strains from prophylactic antibiotic use and failure to control infection by the latent association with viruses and protozoa as well as transmission of microbes by transfusion of blood products. Emphasis should be given to the development of new forms of oral cavity decontamination, new antibiotics to increase the efficiency of intestinal tract sterilisation and measures to sterilize the necessary blood products. Gnotobiotic care must be regarded as an important means of providing supportive therapy for increased efficiency in the treatment of hematological disorders where infection is a potential hazard.

PATIENTS AT RISK

MANY patients who are treated by a hematologist are at high risk of infection. In hematological malignancies the incidence of infection as a cause of death may be as high as 79 % [1]. Impairment of the defense mechanism against infection occurs in a variety of hematological disorders. Therapeutic measures specifically used to treat malignancies or to suppress the immune system before and after bone marrow (or other organ) transplantation increase the risk of infection markedly [2, 3]. Granulocytopenia is one of the most important predisposing factors. In leukemia a high correlation was found between reduced circulating granulocytes and infection, less so between lymphopenia and infection [4]. Drug-induced agranulocytosis often involves serious infection and drug-induced aplastic anemia is complicated frequently by infectious episodes during the course of the disease [5, 6]. A considerable degree of immune deficiency may be present in patients with malignant lymphomas. Most important are immunosuppressive effects of cytostatic and corticosteroids whether unwanted as side effects or specifically used to depress the immune system. The usual pretreatment to condition a recipient for bone marrow grafting does not leave any defense capacity to control microbial infection for considerable time. In addition to granulocytopenia and failure of the immune system, other predisposing factors, subsequent to cytostatic treatment, have to be considered: *e.g.* decrease in number and function of macrophages and lesions of skin and mucous membranes. Diagnostic and therapeutic procedures such as endoscopy, indwelling catheters, and i.v. medication cross the natural protective barriers thus giving way to microbes to invade the body. Conditions that occur in hematological patients causing increased susceptibility to infection are listed in Table 1. It has to be understood that individuals may have several risk factors as in the case of bone marrow grafting in acute leukemia (bone marrow failure and maximal immunosuppression).

NATURE OF INFECTIONS

Ordinarily the individual is associated with a microflora that is composed of various bacteria, fungi, viruses and protozoa. Little is known about the role of extremely oxygen sensitive anaerobes (EOS anaerobes) [7]. Strict and facultative anaerobes, bifidobacteria and bacteroides species compose more than 99 % of the micro-organisms found in the intestinal flora; only 1 % of the gut flora consists of

Table 1. Predisposing factors to infection in hematology

Granulocytopenia:	1 Congenital agranulocytosis (Morbus Kostmann) 2 Drug-induced agranulocytosis 3 Drug-induced aplastic anemia 4 Leukemia and other malignancies involving the bone marrow 5 Bone marrow failure by total body irradiation
Failure of the immune system:	1 Congenital cellular or combined immune deficiency disorders 2 Malignant lymphomas 3 Drug-induced immunosuppression 4 Immunosuppression by total body irradiation 5 Host-versus-graft reaction after bone marrow grafting
Other factors:	1 Lesions of skin and mucous membranes by cytostatics or endoscopy, catheterisation and other diagnostic or therapeutic manipulation 2 Impaired macrophage function in AL or after splenectomy or after cytostatic treatment 3 Impaired bactericidal activity of granulocytes in AL

Enterococci, *E. coli,* Staphylococci, Proteus, Klebsiella–Enterobacter species and yeasts [8]. Pseudomonas species are transitory organisms that are detected in healthy individuals only a short time after ingestion of contaminated food. However, most of bacterial and fungal infections are caused by the above mentioned organisms although they are a numeric minority compared with the total endogenous microflora [10–15]. It is known that abscesses can be caused by bacteroides species [16]. Whether anaerobes are responsible in part of fever of unknown origin in these patients remains unclear [17–20]. Latent infection *e.g.*, in chronic otitis media and teeth granulomas may become the cause of a fatal illness during cytostatic chemotherapy.

Virus infection (*Herpes simplex,* varicella–zoster, cytomegalo virus, and measles) may occur during prolonged and drastic immunosuppression [21]. It may be assumed that these viruses are saprophytic in most individuals but do not cause infection under ordinary circumstances. In contrast, hepatitis virus seems to be primarily transmitted by blood products [1]. The same applies to protozoal infections which are mainly caused by *Pneumocystis carinii* and *Toxoplasma gondii* although epidemic episodes may be observed [22, 23]. Rare infections such as mucormykosis, cryptococcosis, etc. have a higher incidence in the described patient group but nevertheless are infrequent [24]. Also, illness caused by pathogens is less frequent than that caused by potential pathogens. This fact supports the assumption that the "Germ Theory" has considerable limitations [25]. Inoculation, contamination or association does not necessarily lead to infection of the macro-organism (Fig. 1). Infection only occurs when the invading micro-organisms increase rapidly in number and are not localized by the normal body defense mechanisms (Fig. 2). They spread by means

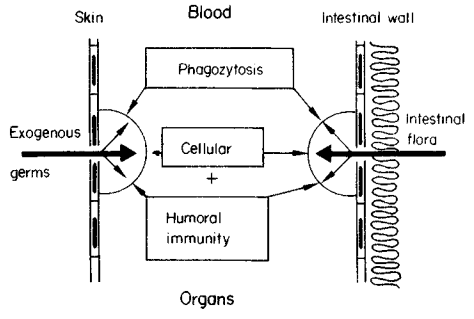

Fig. 1. Localisation of microbes by intact defense mechanism.

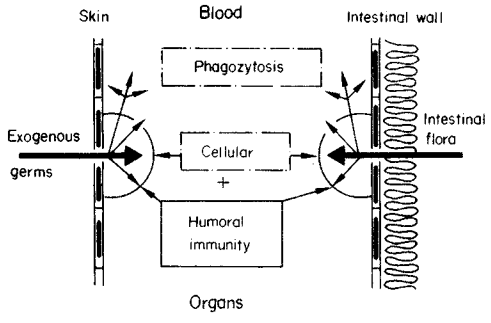

Fig. 2. Microbial invasion in impaired defense mechanism.

of the bloodstream and invade the organs. Most of the saprophytic organisms can become pathogenic under these conditions. Hospitalized patients with acute leukemia have a rapid change in their microflora when detailed culturing of the aerobic bacteria in the fecal specimen is carried out twice weekly [26]. Severely ill patients with various non-hematological disorders show a change in the skin flora when hospitalized [27]. Whether the underlying disease or cytostatic therapy is responsible for this phenomenon remains unknown. Antibiotic treatment of the host, however, causes microbial changes frequently by alterations of the colonization resistance and intermicrobe interaction [28–31]. Antibiotic resistant organisms that are detected in hospitals

more often than in other environments can be transmitted in a number of ways: aerial transport, personnel, instruments, food, processed blood products etc. [32–35].

DIFFICULTIES IN CONTROLLING INFECTION

Even the broad spectrum antibiotics given alone or in combination fail to control infection in a considerable proportion of these patients although doses close to toxicity must be applied [36, 37]. Supportive measures in use are of variable efficiency. Most promising seems to be granulocyte transfusions from compatible donors [38–40]. Adequate numbers of normally functional (*in vitro*) leucocytes can be collected by the use of a continuous flow centrifuge or continuous flow filtration leukapheresis [41–43]. However, the *in vivo* efficiency is not as convincing as the theoretical model suggests when the presentations at the International Symposium on Leucocyte Separation and Transfusion in London 1974 are considered. Present defects of cellular immunity cannot be corrected whereas lack of humoral immunity can be substituted by gamma globulin preparations. Granulocytopenia or only partial restoration of the phagocytic function by the transfusion of donor granulocytes, lack of cellular immunity, change of the intermicrobe interaction and colonization resistance, and multiresistant hospital organisms amount to resulting impairment of the efficiency of antimicrobial treatment. Therefore, prophylactic measures able to prevent association with exogenous organisms and to control the endogenous microflora are used. Standard reverse isolation procedures as practised in hospitals, that is, attending personnel wearing sterile caps, gowns and gloves do not prevent acquisition of potential pathogens [44]. Transmission of microbes by other important routes such as by air and in food is still possible under conventional "isolation" techniques. Utilizing presterilized rooms with filtered air support and aseptic techniques an effective barrier function can be obtained in patient care. Prophylactic therapy with poorly absorbable antibiotics that can be applied in very high doses may suppress the endogenous potential pathogens.

GNOTOBIOTIC CARE

Basic aspects

Gnotobiotic care consists of *strict reverse isolation* to prevent *exogenous* contamination, and *antibiotic decontamination* to prevent invasion of the *endogenous* germs, thus replacing the defense apparatus of phagocytic and cellular immune mechanisms (Fig. 3).

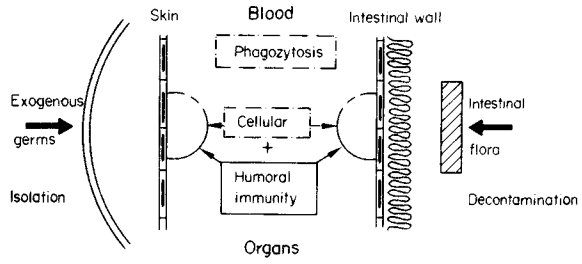

Fig. 3. Prevention of infection by gnotobiotic care.

The word gnotobiotics originates from research in germfree animals indicating that techniques experienced in germfree research have been applied for patient care in medicine. In 1895 Nuttall and Thierfelder could raise germfree guinea pigs obtained by caesarean section [45]. Later, many more animal species have been raised germfree such as chickens, flies, goats, pigs etc. [46, 47]. Experiments have shown that total body irradiation with supralethal doses and toxicity experiments with cyclophosphamide in germfree rats and mice compared with conventional counterparts showed better survival after radiation and better tolerance to the cytostatic drug in the germfree animals [48, 49]. Other experiments demonstrated that grafting with incompatible bone marrow resulted in rapid mortality by graft-versus-host disease in 100 % of conventional mice. Practically all germfree mice survived in satisfactory condition in the same procedure though graft-versus-host reaction was present in animals that were sacrificed and histological sections of reactive organs investigated [50].

States "free of bacteria and fungi" could be achieved in conventional animals by using high doses of poorly absorbable antimicrobial agents under isolation condition. Also, after discontinuation of the antibiotic therapy after several weeks "germfree state" in these animals was observed indicating successful elimination of the endogenous microflora [51]. Further animal experiments of bone marrow grafting with incompatible tissue resulted in complete survival also in decontaminated (ex-conventional) mice as experienced in mice born germfree [52]. Reconventionalization of decontaminated animals after incompatible bone marrow grafting by stepwise inoculation of microbes that belong to the "normal intestinal" microflora and release from the isolation into conventional environment resulted in low mortality [53]. These observations in germfree animals and bacteria and fungi-free ex-conventional animals are the basis of clinical gnotobiology.

Clinical use

The techniques of germfree care are well established in many laboratories since the availability

of plastic isolators [54]. In 1960 they were brought into clinical use by Levenson *et al.* [55]. Subsequently, several strict isolation systems have been designed: the *Life Island* [56], a steel chamber with inbuilt autoclaves and unidirectional air flow [57], *Laminar Air flow rooms* [58–60], *new type plastic isolators* (ULM isolated bed system) providing more comfortable conditions [61, 62] both for infants and adults.

The use of appropriate sterilization techniques ensures optimal reverse isolation. The isolation rooms are sterilized by peracetic acid prior to occupation. Air is filtered by high efficiency filters; all items to be introduced are sterilized either by steam-autoclave or ethylene-oxide sterilization. Only some oral drug preparations and blood products are not sterilizable. Contamination may occur, therefore, despite the strict reverse isolation but significantly less frequent than in patients in other hospital wards [58, 63, 64].

Decontamination concepts aim to render patients germfree. They include surface disinfection, inhalation of antibiotics and the use of large doses of poorly absorbed antimicrobial agents. Significant suppression can be obtained by such measures but only occasionally (and not yet in adults) can a state free of bacteria and fungi be observed [62]. Obviously the difficulty in decontaminating man results from kryptogenic infections such as teeth granuloma, tonsils, and other hidden niches. Indeed, the oral cavity has not yet been successfully decontaminated. Suppression without complete elimination, risks the development of resistant microbes [65]. Thus, the ideal target of gnotobiotic care does not seem to be generally achieved.

Psychological aspects

Psychopathological symptoms occurred in patients who were treated in isolators [66]. Most of these symptoms occurred as a consequence of somatic complication during treatment but were aggravated possibly by the isolation. Patients may adapt psychologically to the isolation during the treatment phase when psychotherapeutic support is given [67, 68]. More patients are reported to have emotional difficulties related to confinement when comparing patients in a *Life Island* system and a *Laminar Air flow room* [60]. In our hands, the confinement in the *Life Island* reduces patient comfort considerably but patient adaptation was much easier and led to the development of fewer emotional difficulties in a newly designed plastic isolation system providing more space and technical aids which can be used by the patient himself (Ulm isolated bed system). No hard data are available to prove the occasionally expressed impression that stay in a Laminar Air Flow Room is less psychologically strenuous than in a plastic isolator.

For infants, long-term observation in plastic isolation systems for more than 2·5 years have shown that with adequate psychotherapeutic care the psychological development takes place within normal limits [69].

Treatment results in hematological disorders

Extensive studies have shown that there is a significantly reduced incidence of infection and mortality in patients with acute leukemia [58, 63, 64, 70]. The remission rate was not better improved in three of the studies, thus missing the original expectations defined by Bodey and colleagues [60]:

Protected environment – prophylactic antibiotic program objective:
Stage 1, Maximally reduce exogenous and endogenous microbial burden;
Stage 2, Decrease risk of infection in patients with AML:
Stage 3, Increase patient tolerance of antitumour agents:
Stage 4, Increase complete remission rates.

In one preliminary publication, a higher remission rate has been reported [64]. In a retrospective study the duration of remission was longer in patients treated in a protected environment/prophylactic antibiotic program [70]. The 100 days survival in a prospective study [58] was significantly longer in patients who were decontaminated and isolated compared to patients who were decontaminated in the usual hospital ward or patients treated with the ordinary hospital care. In our unit we have observed a tendency for longer overall survival of patients who have been treated at least once in isolation or in isolation with decontamination [71]. A prospective randomised study of three patient groups with acute leukemia: isolation and decontamination, isolation and germfree techniques only, and the usual hospital care within the EORTC is nearly complete [72]. In our own unit, preliminary figures (part of the EORTC study) suggest that the rate of complete remission as well as the survival 30 days after the termination of the hospital treatment phase is significantly longer in isolated and decontaminated and isolated only patients in comparison with patients with ordinary hospital care (Table 2).

CONCLUSIONS

Most experience in gnotobiotic care has occurred in relationship to patients with acute leukemia. The

Table 2. Evaluation of gnotobiotic care in the treatment of acute leukemia

Randomisation into three groups:	Patients included: Acute leukemia, aggressive cytostatic chemotherapy to induce remission. Patients excluded before randomization: Age <15 and >60 years, psychological reasons, unwillingness of patients to join the study, creatinine clearance <60 ml/min
A = isolation and decontamination	
B = isolation and germfree techniques only	
C = ordinary hospital care	

				Age (Years)			
	N	1. Therapy	Relapses	Mean	Median	CR	Survival*
A	17	12 (71 %)	5 (29 %)	29·8	29	12 (71 %)	13 (77 %)
B	22	13 (59 %)	9 (41 %)	31·7	26	12 (55 %)	16 (73 %)
C	22	17 (77 %)	5 (23 %)	31·8	26	9 (41 %)	11 (50 %)

*30 days after termination of treatment phase. CR = Complete Remission.

results of several working groups have shown that there is clear reduction of acquisition of new microbes, and of mortality from infection, and that possibly longer remissions, longer overall survival, and higher remission rate are developed. However, a number of problems remain such as incomplete decontamination, possible development of resistant microbial strains from prophylactic antibiotic use and failure to control infection by the latent association with viruses and protozoa as well as transmission of microbes by transfusion of blood products. Emphasis should be given to the development of new forms of oral cavity decontamination, new antibiotics to increase the efficiency of intestinal tract sterilisation and measures to sterilize the necessary blood products. Gnotobiotic care must be regarded as an important means of providing supportive therapy so that increased efficiency will develop in the treatment of hematological disorders where infection is a potential hazard.

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ANALYSIS OF STUDIES ON PROTECTED ENVIRONMENTS AND PROPHYLACTIC ANTIBIOTICS IN ADULT ACUTE LEUKEMIA

Arthur S. Levine, Richard A. Robinson and Janet M. Hauser.

Section on Infectious Disease, National Cancer Institute, Bethesda, MD 20014, U.S.A.

INTRODUCTION

DURING the past decade, twelve major studies have examined the hypothesis that a reduction in the endogenous microbial flora of man, and/or protection from exogenously acquired pathogens, might result in a reduction in infectious disease morbidity and mortality, thus increasing the probability of remission and survival in adult acute leukemia [1–12]. Studies in leukemic rodents have given encouragement to this approach, as it has been shown that prophylactic gastro-intestinal antibiosis and barrier isolation will permit intensive cytotoxic and immunosuppressive treatment that otherwise would be associated with fatal infection, and that intensification of therapy will enhance survival in AKR murine leukemia [13].

The technique of "protective" isolation or "reverse" isolation was common before the antibiotic era. In 1965, a serious effort at reducing infections in patients with neoplasms began at the National Cancer Institute, Bethesda, Md., U.S.A., (NCI) using a large patient isolator system, the "Life Island" [14–18]. It was subsequently found that these units were cumbersome and difficult to maintain, and that the air within often remained contaminated [1]. More recently, it was demonstrated that patient isolation in a protected environment with highly efficient filtration and laminar air-flow did consistently reduce the incidence of microbial contamination in the ambient air and on surfaces [1,10]. Moreover, most patients receiving prophylactic gastrointestinal antibiosis developed stools devoid of culturable micro-organisms [2], although a truly "acquired" gnotobiotic state was not achieved and quantitative anaerobic bacteriologic studies were not reported [2, 4].

The techniques employed in the studies discussed in this analysis include the use of a *Life Island* or *Laminar Air-flow room* (LAFR), topical and orificial antiseptic and antibiotic agents, diets with a low bacterial burden, and bowel decontamination [2, 14–22]. Preliminary studies using all components of a protective regimen [16, 17, 19] or bowel decontamination alone [2], appeared to demonstrate a decrease in the rate of infection. Similar techniques in patients at risk of infection for reasons other than granulocytopenia (surgery, burns and immunologic deficiency syndromes) were also encouraging [23–25]. However, these preliminary studies were conducted without concurrent controls and may not have reflected accurately the contemporary frequency and types of infection in conventionally-treated patients. For this reason, a prospective study was initiated at the NCI in 1968 [1] to determine the efficacy of barrier facilities of an advanced design, and of a comprehensive antimicrobial prophylactic regimen that included gastrointestinal antibiosis. It is the purpose of this report to review the NCI study, to compare and contrast it with similarly controlled investigations, and to present our perception of "the state of the art" in this area of research.

Address for reprints: Arthur S. Levine, Building 10, Room 2B50, National Institutes of Health, Bethesda, MD 20014, U.S.A.

MATERIAL AND METHODS

In the NCI study, we sought to determine the current incidence of severe infection in acute leukemia during chemotherapy of early stages of the disease; the effect on the frequency of infection in such patients when they were given extensive antimicrobial prophylaxis within a protected environment; the effect of suppression of the gastrointestinal flora *per se;* and whether a reduced frequency of infection was associated with an improved antileukemic result. Patients were eligible for this study if they had any morphologic variant of acute leukemia (newly diagnosed or with 1 previous remission), were between 15 and 65 years of age, and were scheduled to receive remission induction chemotherapy. Patients had to be manageable initially in any group of the study, but fever and infection *per se* did not make a patient ineligible. Eligible patients were randomly assigned to receive chemotherapy in the following schemes: protected environment, topical-orificial antiseptics and antibiotics, oral nonabsorbable antibiotics (Gentamicin – 800 mg/daily, vancomycin – 2 g/day, nystatin – 15

million units/day), and a diet low in viable microbial content (Group 1); oral nonabsorbable antibiotics only (Group 2); and conventional ward care with no prophylactic maneuvers (control, Group 3). Randomization was carried out within 24 hr of diagnosis, which was established by bone-marrow examination. Cultures were completed, and prophylactic routines begun within 24 hr after randomization. When a protected environment (PE) was available, patients were randomized, with equal weight between the 3 groups, but if no PE was available, patients were only randomized to Groups 2 and 3.

Patients were "on study" until the following conditions were met: complete remission; evidence of progressive disease after approximately 6 weeks of treatment; or removal of the patient from the isolator unit or the ward. In this study, only severe infections (i.e., septicemia or organ invasion) were considered. Chemotherapy usually consisted of 5-day medication courses alternating with rest periods, and various combinations of vincristine, 6-mercaptopurine, methotrexate, daunorubicin, arabinosyl cytosine, 6-thioguanine, L-asparaginase, and prednisone were used.

The first 5 patients to be randomized to Group 1 were treated within a *Life Island.* Subsequent patients in this group were treated within a semiportable, horizontal *Laminar Air-flow room* (LAFR). All materials entering the protected environments were sterile, except for foodstuffs. Blood cultures were performed when clinically warranted, and the number of blood cultures per febrile episode was comparable in all groups of the study. Statistical comparisons were performed with use of the Fisher–Irwin exact test for 2-by-2 contingency tables, the Wilcoxon rank-sum test and its large-sample analogue, and the generalized Wilcoxon test. The significance probabilities correspond to one-tailed statistical tests.

Comparability of the randomized groups in the NCI study

Patients in all groups were comparable in factors that might influence the incidence of infection. Twenty-six randomizations were made to Group 1, 40 to Group 2, and 30 to Group 3. Patients who died within one week of randomization were excluded from analysis, leaving 22 randomizations in Group 1, 38 in Group 2 and 28 in Group 3. The sex and age distributions of patients in the three groups were similar (mean age of 34 years), and approximately 80 % of the patients in each group were newly diagnosed. About 75 % of the patients in each group had acute myelocytic leukemia. Other factors that might enter into the prognosis of acute leukemia (*e.g.*, an initial peripheral white-cell count greater than 100,000 and fever or documented infection on entry into the study) differed slightly in prevalence, but a statistically significant rate of unfavourable factors could not be discriminated in any one group. Patients spent 47 mean days on study. The percentage of days spent at given peripheral granulocyte levels was similar for each level when Groups 1 and 2 were compared – *e.g.*, these patients spent 28 and 23 % of their time respectively, with granulocyte levels less than 100 per cubic mm. However, control patients spent almost twice as much of their study time with a granulocyte count greater than $1000/mm^3$ than patients in Groups 1 and 2. All three groups received similar chemotherapeutic regimens and dosage schedules. Although there were small differences between the groups in the total dose of any one chemotherapeutic agent, the groups exhibited no overall differences that might influence remission rate or susceptibility to infection or both. In this study, induction chemotherapy was deferred because of myelotoxicity *per se,* and not because of the presence or probability of infection and hemorrhage.

Efficacy of antimicrobial prophylaxis

The maneuvers undertaken in Group 1 were very effective in suppressing the growth of microorganisms in most environmental and patient sites. As a result of the oral administration of non-absorbable antibiotics, together with foods relatively free of viable bacteria, almost all stool cultures revealed no growth of aerobic and anaerobic bacteria, and 79 % no growth of fungi. In Group 2, the same antibiotic treatment with a conventional hospital diet was slightly less effective. Only occasional nausea or diarrhea clearly resulting from the oral antibiotics was noted, and these complications were managed without the need to interrupt prophylaxis in any patient. Noteworthy blood and urine levels of gentamicin were not detected in any patient, even in the presence of renal insufficiency or severe gastro-intestinal ulceration. We did not observe the emergence of a stool organism resistant to gentamicin or vancomycin in either Group 1 or Group 2.

The topical and orificial antibiotic–antiseptic maneuvers appeared to be effective in suppressing the growth of organisms on most skin sites. However, normal flora in the mouth and oropharynx could not be eliminated in any patient, and Gram-negative flora were only rarely eliminated.

Air samples obtained from the *Life Island* and the *Laminar Air-flow room* were compared with samples obtained in a conventional hospital room. With the patient at rest, samples from the protected environments contained fewer than one viable organism per 2·8 m^3, whereas the conventional room contained 400 organisms per 2·8 m^3. During bathing and changing of the bed linens, the air-flow room

contained far fewer organisms than any other situation tested; the *Life Island* was not significantly freer of airborne organisms than the conventional setting. Moreover, 70 % of all air samples in the *Laminar Air-flow room* were sterile, whereas the *Life Island* demonstrated few sterile air cultures during periods of increased patient activity. Approximately 60 % of all surface cultures taken in the air-flow room were sterile while the unit was occupied.

Severe infection during remission-induction chemotherapy

All infections documented at the time of randomization were excluded from this analysis. No difference in the number of episodes of severe infection per patient or in episodes per 100 patient days could be discriminated between the group receiving only oral non-absorbable antibiotics and the control group. However, we documented fewer than 1/2 as many severe infections among Group 1 patients, expressed as episodes per patient or as episodes per 100 patient days. Two episodes of septicemia occurred in Group 1, in contrast to seven such episodes each in Groups 2 and 3. The only episode of septicemia due to pseudomonas species among Group 1 patients occurred in a patient isolated in a *Life Island.* Only one patient in Group 1 had pneumonia, and, in this patient, no organisms were identified in tissue from a percutaneous lung biopsy. In nearly 1/2 of all patients in Groups 2 and 3 pneumonia developed during the study; Group 2 patients also had a relatively high incidence of infections of the skin and mucous membranes. Despite our inability to suppress the growth of fungal organisms completely, fungal infection was no more frequent in either antibiotic-treated group than in the control group.

Among patients whose disease was least responsive to chemotherapy (that is, those with acute myelocytic leukemia) there were 1/2 as many episodes of severe infection per patient in Group 1 as in the other groups. Patients failing to respond to remission–induction therapy had at least twice as many episodes of severe infection per patient in all groups as patients entering remission, but Group 1 patients fared better than the other groups whether or not they had a remission. Group 1 patients also had less total experience with infection in relation to other patients whether or not they had a documented infection at the time of randomization. The differences in frequency of infection between Group 1 and the other groups are particularly significant when life-threatening infections are examined (septicemia, pneumonia and disseminated fungal disease). There were 1/4 as many of these infections in Group 1 as in control patients; again, no significant difference could be discriminated between Groups 2 and 3. We.found that 14 % of all Group 1 patients had one or more life-threatening infections, as contrasted with 37 % of Group 2 and 50 % of control patients.

The groups were further compared for the percentage of days spent on study with severe infection, as a function of various absolute peripheral granulocyte levels. As anticipated, the lower the level, the higher the percentage of days with infection in all groups. With a granulocyte level of less than $100/mm^3$ Group 1 patients spent 12 % of their days with severe infection, Group 2 spent 38 %, and Group 3 spent 40 %. The differences persisted at higher granulocyte levels, but to a lesser degree as a normal granulocyte level was approached. A similar analysis was made of the number of episodes of severe infection occurring per 100 patient-days on study. With fewer than 100 granulocytes/mm^3, Group 1 patients had 1·5 episodes of severe infection, Group 2, 3·71, and controls, 5·45. However, we found no inter-group difference in the mean time until onset of the first severe infection after randomization, nor in the mean duration of established infection. Of the 18 episodes of severe infection in Group 2 in which both an organism and a source could be identified, 1/2 were traced to organisms originally colonizing the an organism and a source could be identified, 1/2 were traced to organisms originally colonizing the oropharynx, and this site correlated well with the high rate of pneumonia in patients given oral non-absorbable antibiotics but otherwise unprotected from exogenous organisms. In control patients, the preponderance of severe infections could be ascribed to organisms colonizing the bowel. Infections that did occur in Group 1 patients appear to have been caused primarily by organisms colonizing areas that proved difficult to decontaminate, such as the perianal region and axillary folds. No infection in Group 1 could be traced to an agent not present at the time of randomization.

Deaths while patients were on study were examined critically; no death in a protected environment could be ascribed to infection in 22 consecutive trials. However, 9 deaths in which infection was the proximate cause occurred in Group 2, and six in Group 3. About 1/4 of all patients in Groups 2 and 3 died during remission–induction chemotherapy, and in virtually all of these cases, infection was shown to be the proximate cause of death. Despite this very substantial reduction in the frequency and lethality of infection among patients isolated within protected environments, no statistically significant intergroup differences were noted in the number of leukemic remissions obtained or in long-term survival. As anticipated, short-term survival was markedly improved in Group 1 patients.

In summary, the results of the NCI study indicated that the use of oral non-absorbable

antibiotics alone did not reduce the frequency of severe infection or the rate of deaths in which infection was the proximate cause. However, patients treated within a protected environment and given an extensive prophylactic antimicrobial regimen had approximately 1/2 as many severe infections and 1/4 as many life-threatening infections as patients treated conventionally. Whereas 22–24 % of patients in Groups 2 and 3 died of infection when receiving remission–induction therapy, no deaths from that cause occurred in the protected-environment group.

Other published or current protected environment and prophylactic antibiotic studies

Our failure to demonstrate the efficacy of oral nonabsorbable antibiotics *per se* stands in contrast to a result reported previously from our institution (NIH

Table 1. Protected environments and prophylactic antibiotics in adult leukemia (Studies with >20 patients)

Study	Result	PE*	PA†	PEPA‡	Comment
Villejuif	INF	+§	ND	ND	Diverse tumors. Controls?
	REM	ND	ND	ND	
Memorial	INF	ND	–	ND	Randomized, but small numbers.
	REM	ND	+§	ND	
Melbourne	INF	ND	+	ND	Retrospective historical matching;
	REM	ND	ND	ND	*S. albus* most frequent isolate in sepsis.
Sydney	INF	ND	+	ND	Historical controls.
	REM	ND	–	ND	
NIH (1970)	INF	ND	+	ND	Historical controls.
	REM	ND	ND	ND	
MDA (1971)	INF	ND	ND	+	Retrospective matching (two controls
	REM	ND	ND	–	differ). Includes consolidation.
MDA (current)	INF	ND	–	+	Randomized, but small numbers;
	REM	ND	–	+	escalation if no infection; PA/SA; no untreated controls.
NIH (1973)	INF	ND	–	+	Randomized, but small numbers;
	REM	ND	–	–	one-tailed statistics; various chemotherapies.
BCRC	INF	ND	+	+	Randomized, but small numbers;
	REM	ND	+	+	remission rates improved for single drug, not for combination.
Roswell Park	INF	+	–	+	Randomized, but small numbers; 25 %
	REM	–	–	–	re-randomized.
Jules Bordet	INF	ND	+	+	Small numbers; random and non-random;
	REM	ND	ND	ND	(*Life Island*).
Ulm	INF	+	ND	+	Small numbers; retrospective matching;
	REM	+	ND	+	(*Life Island*).

*PE = Protected environment
†PA = Prophylactic
‡PEPA = Protected environment + PA
§INF + = Reduced incidence of infection
REM + = Improved remission rate
ND = not done or no data

1970, Table 1), in which it appeared that the antibiotics alone reduced the frequency of infection in leukemic patients as effectively as isolation within the *Life Island.* However, neither the oral-antibiotic study [2] nor the prior *Life Island* study [16, 17] was randomized, and control data were obtained from patients who had been hospitalized up to 10 years earlier. The results of the 1973 NIH study [1] suggest that severe infection and infectious death among leukemic patients treated in this hospital had become significantly less frequent than they were reported to have been earlier in the past decade from the same institution [26]. When experimantal data from the oral-antibiotic study [2] and the *Life Island* study [16, 17] are compared with control data from the present study, the statistical significance of differences is minimized. Thus, it is likely that large numbers of patients must be studied concurrently and at random to reveal unanticipated variables in the complex and evolving situation of nosocomial infections.

This point will become more apparent as we examine another 10 clinical trials reported or in progress which speak to the utility of protected environments and/or prophylactic antibiotics in the therapy of adult acute leukemia (Table 1). All of these trials involved 20 or more patients and were retrospectively or prospectively controlled. Other studies have been reported which concern very small numbers of patients, were not controlled, or which did not examine the treatment of neoplastic disease [16, 17, 22–25].

One of the earliest of the studies described in Table 1 was performed at Villejuif [3]. A large number of initially "pathogen-free" patients with diverse neoplasia or aplastic anemia were treated in an isolated ward containing u.v. lighting and a low-efficiency air filtration mechanism. Prophylactic antibiosis was not employed. A substantial reduction in infectious complications was reported, but the control data was poorly characterized and was not obtained concurrently or at random. No data on remission rates, remission duration, or survival were reported.

Prophylactic antibiosis alone was studied at Memorial Hospital [6], in a prospective, randomized trial of remission induction chemotherapy ("L-6" protocol). Oral non-absorbable antibiotics were employed or not employed in acute non-lymphocytic leukemia. No difference between the groups in any infectious disease parameter was observed; yet the remission rate was 75 % in the antibiotic-treated group and 42 % in the control. In two Australian trials, however, a reduction in the incidence of severe infections with prophylactic non-absorbable oral antibiotics was noted [5, 9]; in the Sydney study, reduction in the incidence of infection was not associated with an improved remission rate. Both Australian studies employed retrospective historical matching. The liability of such a scheme is well illustrated by the finding in the Melbourne study that *S. albus* was the most frequently isolated pathogen in control, but not in experimental patients with sepsis; the authors speculate that this finding may be explained by the observation that the control data were accumulated when indwelling plastic catheters were utilized extensively; at the time of the oral antibiotic trials, scalp-vein needles had replaced indwelling catheters.

Another study which employed retrospective matching as a control was undertaken at the M.D. Anderson Hospital (1971) [10]. The authors point out that in small randomized trials, patients may not be comparable; therefore, the results can be skewed. When all of the variables in a patient population are known, one may avoid this possible skew by matching for such known variables. While this non-random statistical treatment is accurate when all variables can be identified, it seems likely that in the complex situation of leukemia, evolving chemotherapy, and nosocomial infection, all variables are not known. This point is well illustrated by the finding in the 1973 NIH study that control patients spent almost twice as much of their time with a peripheral granulocyte count greater than $1000/mm^3$ as patients in either experimental group, despite comparable therapeutic regimens and dosages in all groups [1]. This finding has not been reported in any other study employing similar antibiosis and chemotherapy. One could not have anticipated such a variable in retrospective matching, and only a large randomized trial would reveal if the result is real or apparent. In the retrospectively matched M.D. Anderson trial [10], two separate control groups were identified as a statistical precaution *vis-à-vis* the method of selection; however, differences exist between the two control groups in regard to chemotherapy response. In this study, experimental patients with ALL or ANLL were isolated in LAFRs or Life Islands and received oral nonabsorbable antibiotics. The experimental group received more intensive chemotherapy than either control group but had fewer infections (statistically significant only for episodes of severe infection at >1000 granulocytes/mm^3). Remissions in the experimental group were longer than in the control groups, and this finding probably relates to the fact that experimental patients were given more extensive chemotherapy since it was believed that the isolation regimen would permit more intensive therapy. Moreover, the difference in remission duration and in frequency of infection might also be explained by the fact that

patients in the protected environment were given additional consolidation treatment after achieving remission. There was no difference between the groups in remission rates in the 1971 M.D. Anderson study.

A randomized study quite similar to the trial at NIH has recently been completed at the Baltimore Cancer Research Center (BCRC), involving 64 non-infected adults with ANLL admitted for their first or second remission–induction therapy [12]. The patients were allocated to LAFRs and oral non-absorbable antibiosis, to normal ward care with oral antibiosis (in 50 % higher doses than in the NIH 1973 study), or to ward care alone. The first two groups experienced a considerable and comparable reduction in frequency of severe infections (provided patients actually ingested the antibiotics), a delay in the onset of the first infection, and a significant difference in remission rate. Yet, the remission rate improvement only occurred in the case of patients receiving daunorubicin alone, and not in those receiving combination chemotherapy. Moreover, among controls receiving daunorubicin alone, only one out of 14 remitted (7 %), a statistic far lower than that reported in any other large trials. Again, it seems probable that small randomized trials, including our NIH study, may exhibit statistical drama but not biological reality.

The prospective study reported from Roswell Park [4] has the same probable defect as the studies from NIH and BCRC, i.e., too small a randomized trial; moreover, almost 1/4 of the patients were randomized more than once. In the Roswell investigation, adults with AML were randomized to receive remission–induction therapy in an isolator (one of four types) without antibiosis, in the ward (conventional reverse isolation) with antibiosis, in the isolator with antibiosis, or in the ward without antimicrobial intervention. A reduction in the frequency of infection after the first three weeks of isolation was found in the isolator, with or without antibiosis, but in contrast to the BCRC result, no effect was noted in the ward group receiving antibiosis. Moreover, there was no improvement in remission rates in any group of the study. Indeed, there were more hemorrhagic deaths in antibiotic-treated patients than in untreated controls (such a result has not been found in other studies).

At the Institut Jules Bordet, adults with acute leukemia received routine ward care (single rooms), prophylactic antibiosis *per se*, or antibiosis in a *Life Island* type of isolator [7]. Some of the patients were randomized, but elderly or psychologically unsuitable patients were non-randomly allocated to the non-isolated study groups. A comparable reduction in frequency of infection at low granulocyte levels was claimed for the antibiotic-only and for the antibiotic plus isolation groups. The authors point out that their inability to effect consistent suppression of gut flora with the antibiotic regimen they employed might relate to their inability to determine whether isolation adds anything to antimicrobial prophylaxis.

At Ulm, the *Life Island* without antibiosis was studied [8]; in this group, and in a group receiving antibiosis plus isolation, there was an apparent reduction in the incidence of infection and an improvement in remission rates, but this study also involved retrospective matching and small numbers of patients.

In an ongoing prospective study at M.D. Anderson [27] adults with ALL or ANLL are randomized to receive prophylactic antibiosis in a LAFR or in the ward; no untreated controls are included. Patients in both groups receive prophylactic oral absorbable and non-absorbable antibiotics (PA) or systemic antibiotics (SA) in a sub-randomization. In the isolation group, there is a mean reduction in the incidence of severe infection and an improved remission rate, in contrast to patients receiving antibiosis in the ward. While the antibiotic *per se* group is not controlled, the incidence of infection and the remission rate in this group are comparable to those usually found when no antimicrobial prophylaxis is practised [28]. In all patients, chemotherapy is given in escalated increments, provided the patient had no infection at previous dose levels. Thus, an attempt is made to answer these questions: Does the LAFR and antibiosis reduce the incidence of infection? Does reduction in the incidence of infection permit more intensive chemotherapy? Does more intensive chemotherapy yield a better overall antileukemic effect? Certain of the data obtained as of this date are described in Table 2. The first patients to be studied received vincristine, cytosine arabinoside, and prednisone (OAP), and later patients received OAP plus adriamycin (AD–OAP). The complete remission rates differ between the protected and unprotected groups for both chemotherapy regimes, the isolated patients showing significantly better results than those receiving antibiosis alone. The reasons for this difference are, however, not immediately apparent. While there are fewer patients infected, fewer fatal infections, and fewer mean days spent with infection in the isolated group than in the antibiotic-only group, none of these differences are sufficiently large to account for the dramatic differences in remission rate. Moreover, because of organ-limiting toxicity other than myelotoxicity, the patients in the LAFRs actually have had the same or less chemotherapy than the patients outside. Once again, one must consider the possibility that the

results to date are influenced by too small a biological sample, even though the statistical sample appears adequate. For example, the distribution of ALL and ANLL patients might differ between the isolated and nonisolated groups; in some reports, the responsiveness of these variants to remission–induction therapy differs [29].

A summary of the effects of protected environments and/or prophylactic antibiotics on the incidence of infection and remission in acute leukemia is shown in Table 3. It can be seen that protective isolation alone (without prophylactic antibiosis) has reduced the incidence of infection in adult leukemia whenever it was studied. However, the result must remain suspect in view of the fact that one of the studies (although randomized) involved a small number of patients, a second study was not controlled, and the third was retrospectively controlled. In one protected environment study, the

Table 2. M.D. Anderson adult acute leukemia escalating induction (Randomized study)

	IN (PEPA, PESA)*		OUT (PA, SA)*	
	OAP	AD–OAP†	OAP	AD–OAP
Patients entered	16	19	26	44
Patients with infection (%)	63	42	73	54
Fatal infections (%)	31	10	42	18
Days with infection at <100 pmn/mm^3 (%)	26	17	42	22
Complete remission (%)	62	84	31	52
Patients–dose escalation (%)	92	47	84	64

* PEPA = Protected environments (LAFR) + prophylactic oral absorbable and non-absorbable antibiotics.
PESA = As above, with systemic antibiotics instead of oral antibiotics.
PA, SA = Prophylactic oral or systemic antibiotics only.
† OAP = Vincristine, cytosine arabinoside, and prednisone.
AD–OAP = Above plus adriamycin.

Table 3. Summary of effects on infection and remission

	PE	PA	PEPA
Infection ↓	Villejuif Roswell Ulm	NIH 1970 BCRC Melbourne Sydney Jules Bordet	Jules Bordet BCRC, Roswell Ulm MDA 1971 MDA current NIH 1973
Infection N.C.*		Memorial Roswell MDA current NIH 1973	
Remission rate ↑	Ulm	Memorial BCRC	MDA current BCRC Ulm
Remission rate N.C.	Roswell	Roswell MDA current NIH 1973 Sydney	Roswell MDA 1971 NIH 1973

*N.C. = No change.

remission rate was improved in association with the apparent reduction in the incidence of infection, but in a second trial, there was no change in remission rate. A larger total number of patients has been examined for the effect of prophylactic antibiosis *per se;* half of the studies exhibit a reduction in the frequency of infection, but half exhibit no change. Moreover, of the apparently well-controlled studies which examine the effect of antibiosis alone on remission rates, two show improvement, (Memorial, BCRC) and two show no change, (Roswell, NIH 1973). The largest number of patients studied are those receiving prophylactic antibiosis in a protected environment. At least seven such studies have shown a reduction in the incidence of infection, and none has failed to demonstrate this benefit. However, two prospectively randomized studies (BCRC, M.D. Anderson current) show an apparently improved remission rate in association with the reduction in the incidence of infection, and two studies of comparable design (Roswell, NIH 1973) show no such effect on the remission rate.

DISCUSSION

What conclusions can be drawn from this decade of research? It may well be that differences in remission–induction rate, survival, and incidence of infection are tied intimately to the specific form of antileukemia therapy employed, and that benefits from antimicrobial manipulations specifically depend on the variant of acute leukemia, the age of the patient, and unknown variables. These specific relationships may have been obscured in certain of the studies described in Table 3 but not in others, thus precluding consistent results. Nonetheless, it seems likely that sufficient data exist (Table 3) to permit certain generalizations: (1) The combination of prophylactic antibiosis and efficient isolation–air filtration appears to reduce the incidence of infectious morbidity and mortality in adults receiving remission–induction therapy for acute leukemia. This result is obtained because of the exponential reduction in both endogenous flora and pathogens ordinarily transmitted by the air. The consistent degree of reduction in the frequency of infection can be determined only after a further large randomized trial involving the same type of effective isolator and antibiosis.(2) The effect of prophylactic antibiosis *per se* on the incidence of infection is not obtained consistently or is less than that of antibiosis in a protected environment; the probability of selection

*Table 4. Cost of a four-unit Laminar Air-flow facility**

Initial costs	
Four LAFRs @ $20,000	$ 80,000
Additional supplies @ $750 per LAFR	3,000
Gas sterilizer	10,000
Special diet kitchen equipment	21,000
Architectural/engineering service/remodeling	20,000
	$134,000
Additional staff	
2 RNs @ $9,500	$ 19,000
1 LPN @ $7,000	7,000
2 supply clerks @ $5,000	10,000
1½ dietary aides	7,500
½ dietitian	4,500
1 laboratory technician	9,000
	$ 57,000
Additional daily staff costs	
Assuming an 80 % occupancy	$50/patient day
Assuming a 50 % occupancy	$85/patient day
Daily supply costs	
Sterile supplies and bacteriology supplies	$100–$150/patient day
Prophylactic antimicrobial agents (including oral non-absorbable antibiotics)	$50–$100/patient day
Total daily costs of staff and supplies	$200–$300/patient day

*Most of these expenses are in addition to the expenses of conventional hospital care.

for resistant organisms is sufficiently greater in the ward than in isolation (with a continuing large inoculum of ambient microorganisms in the ward) [7, 11] that antibiosis should not be further studied outside of a barrier facility. With such a facility, there is also less likelihood of spread of resistant organisms to the community at large.(3) While some data suggest that the protected environment alone (without antibiosis) may be of value, these data are scanty and lack a theoretical foundation since patients with hematologic malignancy appear to have sepsis of enteric origin as frequently as pneumonia [28].(4) There may be an improvement in the remission rates of adult acute leukemia in association with a reduced frequency of infection, but this difference must be small or it would already have been consistently demonstrable. It is evident that many tumors remain drug-resistant whether or not the patient becomes infected. Consequently, only a subset of the total population with acute leukemia may fail to have a remission because of supervening infection. It is also apparent that certain areas of the body cannot be effectively decontaminated and that some patients still become infected within the protected environment. For example, after the completion of the 1973 study, we began a trial of cytosine arabinoside plus 6-thioguanine induction in adult AML. Four patients in the LAFR, receiving oral non-absorbable antibiotics, were given anti-leukemia therapy to the point of bone marrow aplasia and for several days beyond that point in an attempt to induce more enduring remissions. Three of the four patients died of sepsis and one remitted; the study was terminated (Levine, Robinson, Hauser, and S. E. Siegel, unpublished results). Finally, some patients undoubtedly experience remission of their disease before possible infection. For these reasons, the ultimate utility of protected environments and prophylactic antibiotics in the therapy of acute leukemia remains uncertain. To demonstrate a small (*e.g.*, 20 %) difference in remission rate between controls and experimentals will require a further large randomized study (150 patients in one institution receiving the same chemotherapy for the same disease). One must then ask if the cost-benefit of such trials is justifiable, in view of the expense of such studies (Table 4) [30].

An answer to the question of cost–benefit likely will be negative if one is only to consider the significant enhancement of remission rates in adults with acute non-lymphocytic leukemia. However, there are a number of other disease states in which the patient is at risk of endogenous and exogenous infection (*e.g.*, aplastic anemia, immunologic deficiency syndromes, organ transplantation, and other neoplasia). The results of protected environment-prophylactic antibiotic studies in patients with leukemia might therefore be extrapolated to an aggregate of patients whose long-term survival is primarily compromised by infection, and be justifiable as an experiment in human gnotobiology, even though an improvement in long-term leukemia survival may not be large nor consistent at this time.

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NEUTROPHILS COLLECTION AND TRANSFUSION FOR THE TREATMENT OF INFECTION IN NEUTROPENIC PATIENTS*

P. Stryckmans and L. DeBusscher

Service de Médecine Interne et d'Investigation Clinique de l'Institut Jules Bordet, Brussels, Belgium.

IN 1953 Brecher, Wilbur and Cronkite [1] demonstrated that homologous neutrophils transfused to irradiated dogs were able to circulate and to migrate into inflammatory sites. The application of this observation to the human took several years. The limiting factor was a technical one; only small quantities of granulocytes could be collected from normal donors. The first step for the clinical application of neutrophil transfusion was the use of white cells from patients with chronic myeloid leukemia [2, 3]. Then, in 1963 the introduction of the NCI-IBM continuous flow blood cell separator [4] made it possible to collect, for the first time, greater amounts of white cells from normal donors. Many improvements of continuous flow centrifugation (CFC) have since been described. Finally, in 1970, a method of collection, simpler and cheaper, continuous flow filtration leukapheresis (CFF), was introduced [5].

During the last decade, a considerable amount of work has been performed in many centers using these techniques. It should be possible by now to evaluate, from all the published or presented data, the usefulness of granulocyte transfusions in the treatment of infected neutropenic patients. Two criteria to evaluate this procedure can be used: first, the level of white blood cells of the recipient after transfusion (the 1 hr-post-transfusion increment) has been used by most investigators, second, the effect of polymorphonuclear cells (PMN) transfusions on morbidity and/or mortality of infection in neutropenic patients.

The increment of circulating PMN found in the recipient after PMN transfusion depends on: (a) the number of PMN transfused or collected; (b) the percentage of injected PMN which is recovered in the circulation.

(a) The number of PMN collected

(1) The factors which increase the circulating PMN of the donor. Physiological mechanisms can be exploited in order to increase the number of circulating PMN of a normal individual.

(i) The normal egress of circulating PMN to the tissues can be hampered. This will result in prolongation of the transit time of the PMN in the circulation.

(ii) The normal influx into the circulation of PMN from the bone marrow reserve can be increased.

(iii) The normal exchanges between blood-marginating and blood-circulating pools can be modified in favor of the latter.

(iv) The return of PMN from the tissues to the peripheral blood, although theoretically possible, seems improbable [6].

Etiocholanolone, a metabolite of dehydroepiandrosterone, increases the leucocyte count by stimulating the release of mature PMN from the bone marrow reserve into the circulation [7, 8]. A mean maximum PMN increase of 8–9000/mm^3 is obtained within 12–15 hr after an i.m. injection of 0·2 mg/kg [7]. This agent, at the dose of 4 mg/m^2, made it possible to increase the collection of granulocytes with the blood cell separator [9].

Corticosteroids have been shown to act by simultaneously increasing the output of PMN from the bone marrow reserve and decreasing the egress of PMN to the tissues [10]. The possibility cannot be excluded that corticosteroids also trigger the return of some tissue PMN to the circulation [11]. One injection of 100 mg hydrocortisone i.v. within 3–5 hr produced an average increase of the circulating granulocyte pool of 130 % [10]. In order to prepare them, some authors therefore administer 100 mg of hydrocortisone hemisuccinate i.v. [12] to donors 2

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Address for reprints: P. Stryckmans, Institut J. Bordet, 1, rue Héger-Bordet, 1000 Brussels, Belgium.

hr before the beginning of PMN collection, 50 mg at the beginning and 25 mg each hr during collection. Others administer dexamethasone (4 mg/m^2) by mouth within the 12 hr immediately before the leukapheresis [13]..

The procedure of CFF by itself after a transient neutropenia, [14] induces a leukocytosis [15] which probably results from a bone marrow release by a mechanism which is not yet known. Physical exercise is known for long to induce an increased leukocyte count by increasing the blood flow and thereby releasing in the circulating pool, the marginating PMN adhering on, or rolling along the inner surface of the small blood vessels [16]. The possibility of preparing PMN donors by physical exercise has been reemphasized recently [17]. The authors found a more than 100 % increase of circulating PMN after 2 hr of exercise. An additional increase could be obtained by the simultaneous use of hydrocortisone (200 mg, i.v.).

(2) The method of PMN collection (Table 1). The first principle of separation of PMN used has been sedimentation of the RBC through the simple use of plastic blood bank bags. This procedure is too slow and is therefore useful only on blood from donors with CML and high WBC counts (100,000–200,000/mm^3). The yield of this simple procedure, with the addition of macromolecules (Plasmagel) in order to increase the red cell sedimentation rate, is of the order of 90 % for CML blood [18]. The number of leukocytes collected from a CML donor with 200,000 WBC/mm^3 is therefore of the order of 8 x 10^{10}/hr. In many centers, the blood of CML patients is used routinely for transfusion to neutropenic patients. In these centers, CML patients are often treated with hydroxyurea, an agent whose effect, being rapidly reversible, permits a return to high WBC counts within a few days after cessation of therapy.

The second principle of separation, namely centrifugation, became useful only when it could be applied on donor blood flowing continuously (25–50 ml/min) in an extracorporeal circuit [4, 19]. The yield of the procedure is still low, of the order of 20 %, indicating that approximately only 1 PMN out of 5 can be trapped and collected. The geometry of the centrifuge bowl is certainly an important factor for the efficiency of the procedure. A great effort is presently made in this direction. The speed of rotation is certainly another important factor. With an IBM blood cell separator, the yield increased from 22 to 46 % when the speed was decreased from 625 to 450 rev/min [20]. An additional way to increase the yield is through the use of macromolecules which increase the sedimentation rate of the red blood cells. Hydroxyethyl starch (HES), a non-antigenic agent (glucopyranose polymer) [9], and Plasmagel ® (a solution of modified fluid gelatin) [12, 20] have been advocated. With the latter, a yield of 50 % could be obtained. The highest reported mean PMN collection /hr using centrifugation is 0·9–0·7 x 10^{10}. This could be obtained when Plasmagel and hydrocortisone were used simultaneously [12] or HES and etiocholanolone simultaneously [9].

The third principle of collection has been introduced by Djerassi [5] and depends on the capacity of the PMN to adhere, and to adhere reversibly, to nylon fibers. Blood is collected by venipuncture and passed through 4 Leukopak filters in parallel; the whole blood is returned to the donor and the PMN are eluted from the nylon filters. The yield appears to be of the order of 65 % [21]. Up to 1–2 x 10^{10} PMN can be collected per hr by this procedure (Table 1). The advantages of this technique are its simplicity, its low cost, and its efficacy.

(b) The recovery of the transfused PMN in the recipient

(1) *Evaluation of the fate of transfused PMN.* From autologous transfusions [16, 22], it is known that cells can either (i) marginate on the wall of capillaries and post-capillary veinules; normally, approximately 50 % marginate within a few minutes, (ii) leave at random to the tissues; normally 50 % leave the circulation within 6–7 hr, (iii) become pyknotic and die; a very small fraction become pyknotic in the blood within 24–30 hr [23], the majority thus die in the tissues after a life span which is still completely unknown. In the case of homologous transfusion, these processes probably occur in a more or less normal fashion but an unknown fraction could also be sequestered definitely or reversibly for an unknown period of time as was seen at least for CML cells [24].

One way to evaluate margination and eventual sequestration of PMN transfused is the one-hour-post-transfusion PMN increment. In order to make it comparable from patient to patient this parameter is now expressed per m^2 of recipient BSA per 10^{10} PMN injected. This parameter was found to be 590/mm^3/m^2/10^{10} for PMN collected by CFC and 66 for PMN collected by CFF in a strictly controlled study [25]. The difference could be due to increased stickiness [26] of CFC collected PMN and therefore increased tendency of these PMN to marginate [27].

Another way to express the result of PMN transfusion is the 1-hr-post-transfusion recovery (the number of circulating PMN in % of the number

Table 1

		Sed. in CML	CFC	CFC modified	CFF
Donor	ESR accelerator	Plasmagel®	0	Hydroxyethyl starch or Plasmagel®	0
	Leukocytosis inducing agent	0	0	Corticosteroids or etiocholanolone	Corticosteroids
Procedure	ml donor blood processed/hr	400	2500	2500	2500–5000[21, 40]
	Yield (in %)	90[18]	~20	~50	65[21, 40]
	1×10^{10} PMN collected/hr	8	0·1	0·7[9] 0·9[12]	0.47[15] 2[21, 40]
Recipient	1 hr post transfusion recovery	4·8 %[2]	0–75[28]		
	Corrected PMN 1 hour increment	–	590[25]	–	66[25]
	PMN T 1/2 (in hr)	24[2]	6.5[49]	–	9–15[27]

of transfused PMN). The mean value was very low, (4·8 %), when PMN from CML patients were transfused [2]. When normal donors were used, this value was found to vary between 0 and 75 % [28]. These variations were apparently related to the degree of compatibility between donor and recipient suggesting that these early measurements do not only evaluate margination but also sequestration and early cell death.

A different information can be obtained from the half-time (T 1/2) of the transfused PMN which remain in the circulation 1 hr after transfusion. A prolonged T 1/2 was found to be correlated very well to a decreased capacity of the cells to egress to inflammatory sites. Only scarce information exists on homologous PMN transfusion in humans; the T 1/2 appears to be normal (6–7 hr) for CFC–PMN [28] and to be prolonged (9–15 hr) for CFF–PMN [27].

Migration to the tissues and especially to inflammatory sites within 1–2 hr has been observed by several authors. However, quantitative studies are needed in order to evaluate the roles of compatibility and of the different collection procedures on the capacity of the transfused PMN to reach inflammatory sites.

(2) *Factors affecting transfused PMN recovery.* In our series using related and random unrelated volunteers, an average 16 % recovery 1 hr post-transfusion was observed as in most studies. Graw *et al.* [27] studied the average PMN recovery in the recipients classified in 4 groups made according to the matching of the 4 HL-A antigens of the donor and the recipient. This is shown on Table 2 made according to their results. Other publications [29] also suggest that the HL-A antigen system could be a major factor for PMN compatibility in terms of PMN recovery. Unfortunately, the chances of having a random unrelated donor identical in the HL-A system is very low. Only siblings have a 25 % chance of being identical. In practical terms, the chances of having a matched donor depend on the number of siblings one has. Thomas [30] found in his series that 41 % (110/263) of the patients had an HL-A identical sibling for BM transplantation. We calculated that in

Table 2

HL-A matching and the 1 hr post-transfusion PMN recovery in %

If matched for	4 HL–A antigens	~50 %
for	3 HL–A antigens	~27 %
for	2 HL–A antigens	~17 %
for	1 HL–A antigen	< 5 %

Made from the work of Graw *et al.* [28].

our institute, the chances were only 7·5 % (14/177) for an adult or an elderly to have an identical HL-A sibling for PMN transfusion.

On the other hand, it should be stressed that the response to PMN transfusions, in terms of clinical efficacy, in those patients who has bacterial or fungal infections resistant to antibiotic therapy appeared to be unrelated to HL–A compatibility [31].

The presence of leukoagglutinins in the recipient's plasma is another important factor to consider. Their specificity is unknown. Their incidence in general seems to increase with the number of transfusions and with the degree of incompatibility in the HL–A system but not always [32]. Moreover, they are also encountered in normal untransfused individuals and in HL–A compatible pairs suggesting that antigen differences other than HL–A may play a role [32]. Their presence should be recognized because they may be responsible for febrile reactions, severe dyspnea and transient pulmonary infiltrates within 24–48 hr after a WBC transfusion [33]. They were found to be associated with low post-transfusion recoveries and decreased antimicrobial activity [34]. On the other hand, some authors consider that their presence was not associated with a decreased clinical response to the PMN transfusion [31].

Compatibility in the ABO system has also been found to affect considerably the 1-hr-post-transfusion recovery. Indeed, a mean recovery of 50 % was observed when HL–A and ABO were matched, whereas only 7 % was recovered when HL–A matched-ABO mismatched transfusions were given [28]. Whether this indicates the expression of ABO antigens on the surface of the granulocyte [35] or the deleterious effect on PMN of the presence of mismatched contaminating red cells was not clear.

This brings up the possible influence on the recovery of PMN of other cells "contaminating" the PMN transfusion. The severe neutropenia which has been sometimes seen for 1–4 days after a transfusion of HL–A mismatched platelets [36] suggests that HL–A matching may at least be indirectly related to the PMN efficiency. The role of immunization of the recipient by previous transfusions is suggested by an increasing frequency of leukoagglutinins when the number of PMN transfusions increases [31]. This observation contrasts with another one indicating that sequential transfusions of CML–PMN always gave a recovery of the order of 5 % [2]. The infectious status of the recipient seems also to play a role since a higher recovery was observed in afebrile patients than in febrile patients [2]. This suggests the rapid "utilization" of PMN in infected patients. Spleen size, which appears to influence autologous post-transfusion PMN recovery [37], has hitherto not been investigated after homologous PMN transfusion.

(c) *The functional capacity of the collected PMN*

PMN collected by simple centrifugation (without additives) appear to be practically normal for most tests performed so far. For CFF–PMN, electron microscopy has shown cytoplasmic vacuolization, mitochondrial swelling [38] and clumping of glycogen particles [39]. These morphologic abnormalities were rapidly reversible after washing and resuspension of the eluted cells with heparinized plasma or tissue culture media [39]. Moreover, impaired viability and function were described for PMN collected by CFF [15]. On the other hand, in a recent publication, the phagocytic, metabolic (^{14}C–I–glucose oxidation and protein iodination) and chemotactic properties of CFF–PMN were found to be equivalent to those of control cells obtained from the same donors prior to CFF [40]. These discrepancies between results obtained in different centers were attributed to differences in eluting solution, eluting pressure and centrifugation speed after elution [40].

Another point which deserves attention is the use of corticosteroids to prepare the donor. It is known that hydrocortisone seriously blunts the inflammatory response [41], interferes with mobilization of PMN into areas of tissue damage [42], suppresses phagocytosis [43] and diminishes lysosomal release [44]. It is therefore crucial to know whether the "steroid lesion" is reversible and, if it is, how long it lasts after the PMN has been removed from the steroid-treated individual. A partial answer to this question exists since it was found that PMN collected by CFC from donors prepared by a single i.v. injection of hydrocortisone (120 mg/m^2 BSA, 2 hr prior to initiation of a 4 hr leukapheresis) showed normal phagocytosis and bactericidal activity *in vitro* [45].

X-ray irradiation (1500 R) of all random donor–WBC transfusion is performed in many centers in order to avoid graft-versus-host reactions in the recipient (see below). This dose of X-ray has been found not to alter the function of PMN *in vitro* [46, 47] while at the same time decreasing the response of lymphocytes to phytohaemagglutinin [46]. On the other hand, it was said to decrease the 1 hr post-transfusion recovery (not the 15 hr recovery) and to reduce the clinical efficiency of the transfusion. Sixty per cent of the patients responded clinically to non-irradiated PMN whereas only 43 % responded to irradiated PMN [47].

(d) Hazards of PMN transfusions for the recipient

Two complications have been described which seem to occur mainly when CML white cells are transfused to immunosuppressed patients. The first has been the development in the recipient of digestive and/or cutaneous symptoms reminiscent of the secondary disease seen after injections of allogeneic bone marrow. Out of 33 transfused patients, these symptoms, probably related to a graft-versus-host reaction, appeared in 7 who received at least 10^{11} CML white blood cells [3]. The symptoms were reversible in 6 patients but fatal in one. This complication was often found associated with the second one, namely the ability of some myeloid blood cells from CML to engraft in the bone marrow. This engraftment was usually temporary. The longest graft reported lasted for 79 days [48]. One case described by Graw *et al.* [49] died 25 days after 8 transfusions of CML blood. Cytogenetic studies and necropsy suggested total repopulation of the patient's bone-marrow with CML cells. More recently, the case was described of an immunosuppressed patient, who 28 days after a single transfusion of $3{\cdot}3 \times 10^{11}$ CML leukocytes, had 180,000 WBC/mm^3 in the blood, a bone marrow diagnostic of CML and the Ph' chromosome of the donor in 85 % of the cultured bone marrow cells. The patient died at day 44 post-transfusion with an hematological picture of active CML [50].

Chills and fever appear to be the main inconvenience of transfusions of PMN collected by CFF. This complication was encountered in 8 patients out of 16 receiving CFF–PMN while it was never encountered in the same pairs of donor-recipient when CFC–PMN were administered [25]. The high incidence of this complication has been confirmed by several authors [51, 52].

(e) The effect of PMN transfusions on the infectious status

Three parameters can be investigated: the infectious morbidity, the infectious mortality and the negativation of bacteriological cultures.

(1) *Animal experimental results.* Several studies on animals suggest that PMN transfusion might be extremely useful in patients. Three salient studies are summarized in Table 3. The first [53] indicates the protective effect of one PMN transfusion on neutropenic, infected dogs. This study does not cover the usefulness of PMN transfusions in the presence of neutropenia when the infection is treated by the most adequate antibiotic(s). The second study [54] indicates, with a similar experimental system, that PMN collected by centrifugation were helpful even when the animals were treated simultaneously with high doses of gentamicin which was shown to be very active *in vitro* against the infecting agent used. One remark concerning this study is that the Pseudomonas used to infect the dogs were injected intrabronchially. This route of administration caused pulmonary infections which could have been preferentially improved by transfusions due to possible pulmonary PMN sequestration. The third study [55], methodologically identical to the second, confirms that PMN associated with gentamicin are equally effective whether they are collected by centrifugation or collected by filtration [56]. It seems to indicate,

Table 3. Results in neutropenic animals

Experim. system	PMN /mm^3	Infection	Antibiotics	No. Tx	Median survival (days)	P	References
1200 R Irradiated dogs	<1000	*E. coli* at day 4	0	1	2·7	<0·01	[53]
			0	1	3·7		
350 R Irradiated dogs	<1000	Pseudom. at day 6	Gentamicin	0	4	<0·05	[54]
			Gentamicin	≥7*	13		
350 R Irradiated dogs	<1000	Pseudom. at day 6	Gentamicin + Carbenicillin	0	13·5		[55]
			Carbenicillin	0	11		
			Gentamicin	0	4	<0·05	
			Gentamicin	≥7†	25		

*CFC; † CFF; No. Tx: number of PMN transfusions.

moreover, that the PMN might not add as much to carbenicillin as to gentamicin. It should be reminded in this respect that aminoglycosides are generally considered as agents needing the collaboration of PMN to be active *in vivo* [57]. Gentamicin might, for this reason, not have been the most suitable agent to associate with PMN transfusions in order to demonstrate the additional effect that PMN could eventually bring by themselves.

(2) *The effect of PMN transfusion on infected neutropenic patients.* Most studies published or reported so far on this topic can be classified in 3 main categories: a, b or c of Table 4, according to the indications used to administer PMN transfusions. In the 3, antibiotics (AB) were given but their adequacy was different by definition. The results published so far concern one of these 3 categories. They will be analysed separately.

(a) Effect of PMN transfusions and empirical AB at the onset of fever

It is shown in Table 5 that in 3 studies [20, 58, 59], the mortality by infection was similar for transfused patients and non-transfused patients taken as reference. The main criticism to these studies still in progress is the relatively low number of transfusions, and the low number of PMN's per transfusion. It is unlikely that the negative answers seen after using this indication for PMN transfusion would result from the presence of micro-organisms different from those encountered with other indications since the response rates were found similar regardless of the infecting agent [60].

(b) Effect of PMN transfusion on fever persistent despite AB

Table 6 summarizes the clinical results observed after PMN transfusion [2, 3, 12, 31, 61, 62] given in this way. The criteria of improvement were generally temperature lysis within 12–36 hr and, in some studies, clearing of infection clinically and on X-ray films and disappearance of the infecting organism from cultures. Details on the criteria of evaluation, except for fever, are generally not indicated.

Table 4. PMN Tx and antibiotics to neutropenic patients (<500 PMN)

	Indication for PMN	Adequacy of antibiotics	Reason for delay in PMN Tx
(a)	At onset of fever (38^5) or infection	Unknown	Operational ~24 hr
(b)	After persistence of fever or infection despite antibiotics	No	Evidence of resistance >48–72 hr
(c)	When Gram-neg. septicemia was proven	Yes	Bacteriological ⩾24 hr

Moreover, as shown in Table 6, in only one of these studies were there non-transfused patients taken as reference. In addition, it is not indicated whether in these patients, neutropenic at the beginning of the study, autologous PMN seemed to reappear during the period of evaluation of the effect of transfusions. This is important since as soon as autologous PMN production resumes, it is evident from the observa-

Table 5. (a) Effect of PMN Transfusions (Tx) and empirical antibiotic at the onset of fever

Indication	Method collection	No. PMN x 10^{10}	No. Tx	No. Tx treated infections	% Survivors Tx Group	% Survivors Control group	References
<500 PMN >101 F	CFC	0·7	4	23	78	80 (C)	[58]
<500 PMN >38^5 F	CFC	1	2	11	100	100 (R)	[20]
<250 PMN >101 F	CFF	1–5	4	12	86	82 (R)	[59]

(C) Control group made of patients for whom a PMN donor could not be found
(R) Randomized study

tion of non-transfused neutropenic patients, that lysis of temperature often occurs. For all the reasons indicated above, the real incidence of improvement in all these studies is difficult to estimate. The most constant observation is certainly that after greater number of transfused PMN, apparently higher percentages of improvement are encountered [2, 3, 12, 61]. It thus appears to be an essential principle in the field of PMN transfusion to transfuse the greatest amount possible. The last study on Table 6 indicates that mortality due to infection might be decreased by PMN transfusions. However, due to the small number of comparable patients, this randomized study still leaves some doubt [62].

(c) The effect of PMN transfusions on neutropenic patients (<500 PMN/mm^3) with proven Gram-negative septicemia treated with adequate antibiotics [25, 28] (Table 7).

These studies strongly suggest a decreased mortality when at least 4 PMN transfusions are given. However, due to the fact that the reference undata also remain inconclusive and require confirmation. Randomized studies are presently in progress in order to elucidate the role of PMN transfusions in septicemic neutropenic patients.

It is important to draw attention to the fact that for these 3 indications, a delay of at least 24 hr existed between the onset of fever and the first PMN transfusion. This delay is technical (donor availability, time for collection and irradiation, . . .) for indication (a), implied by the clinical definition of indication (b) and by the bacteriological definition of indication (c). In this regard, it is noteworthy to recall that a high proportion of death by infection in these neutropenic patients occurs soon after the clinical onset of the infection. As a matter of fact, among 69 neutropenic (<1000 PMN/mm^3), febrile (>38°C) patients treated at the Institut J. Bordet according to the EORTC antimicrobial project, 15 died within the period of evaluation, i.e. the first week of their infection. Autopsy and bacteriological results indicated that 5 did not die from infection, that 5 probably did not die from infection and 5

Table 6. (b) Effect of PMN transfusions (Tx) on fever persistent despite antibiotics

Donors	PMN collection method	Amount WBC $10^{10}/m^2$	No. Tx treated infections	% improved* Tx patients	Results in non-Tx patients	References
CML	Sedim.	<10	14	35	?	[3]
CML	Sedim.	>20	30	66	?	
CML	CFC	2–15·5	81	54	?	[2]
CML	CFC	15·6	11	100	?	
N	CFC	1·2–4·5	202	66	?	[31]
N	CFC	2·5–5	13	61	?	[12]
N	CFC	>5	7	100	?	
N or CML	CFC	–	72	40	?	[61]
N	CFF	>6	17	88†	26†(R)	[62]

*Temperature lysis within 12–36 hr was the only criteria used in all the studies.
†% Survival
(R): randomized study.

Table 7. (c) Effect of PMN transfusions (Tx) on neutropenic patients (<500 PMN/mm^3) with proven Gram-neg. septicemia receiving adequate antibiotics

Collection technique	No. PMN x 10^{10}	No. Tx	No. patients transfused	% Survivors Tx Group	% Survivors Ref. Group	References
CFC	0·1–4·2	1–14	39	46	30(C)	[28]
CFC	0·1–4·2	4–14	12	100	26(C)	
CFF	0·4–4·8	4–30	9	56	–	[25]

(C) Control group made of patients for whom a PMN donor could not be found.

almost certainly did die from infection. Fig. 1 shows that among the ten who probably or certainly died from infection, 6 died within the 24 hr following the beginning of treatment. Thus, this indicates that even with indication (a) consisting in the earliest administration of therapeutic PMN transfusion, 3 out of the 5 patients who certainly died of infection would not have been saved by these transfusions due to the technical delay inherent to the technique presently in use.

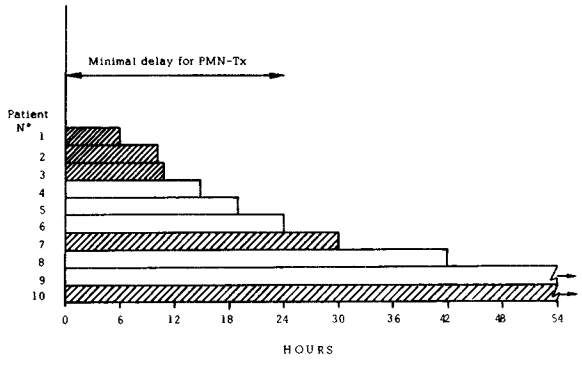

Fig. 1. Survival in hours from the onset of fever (>38° C) and beginning of antibiotics of 10 neutropenic patients who certainly (▨) or possibly (□) died of infection. These 10 patients belong to a group of 69 neutropenic infected patients treated by A. B. according to the protocol of the EORTC antimicrobial project.

The high incidence of death at the very beginning of infection in neutropenic patients raises the possibility of using 2 other transfusion modalities. The first is having PMN immediately available. It implies the difficult problem of PMN cryopreservation. Attempts are made in this direction but no clinical evaluation of cryopreserved PMN transfusions has so far been possible. The second modality concerns another indication of PMN transfusions; namely their prophylactic injection into neutropenic, non-infected, afebrile patients. Theoretically, relatively small numbers of PMN might logically be more effective against incipient infection than against advanced infection. Practically, higher PMN blood counts can be maintained in non-infected patients than in infected patients for same number of PMN transfused. However, no conclusive clinical results exist so far to recommend prophylactic PMN transfusions.

CONCLUSION

Techniques are presently available for obtaining from normal donors, per day, quantities of PMN equivalent to 1/3–2/3 of the total number of PMN produced per day by one normal adult. The collecting procedures apparently do not affect the viability and the function of the PMN's.

Controlled studies in animals indicate that PMN transfusions are helpful since they increase the survival of infected neutropenic animals treated concommittently by antibiotics.

There are several studies in humans also, which suggest that infectious morbidity and mortality can be decreased by PMN transfusions; but all of these studies hitherto failed to demonstrate it conclusively. This is due either to absence in these studies of reference non-transfused patients, or to the insufficient number of evaluable patients due to the heterogeneity of the groups as to the clinical situation of the patients. There are, on the other hand, studies in which no effect of PMN was found. These studies also could not prove, in this case, the inefficiency of PMN, either because too small amounts of cells were transfused, or because the concomittant antibiotherapy used was already very efficient by itself.

The exact place of PMN transfusions in the armamentarium against infection in neutropenic patients has still to be indicated by methodologically perfect studies. Such studies, to be demonstrative will probably require: (1) the random allocation of the treatment (PMN plus antibiotics or antibiotics alone); (2) the administration of large amounts of PMN 2·5 x $10^{10}/m^2$ body surface area given at least 4 consecutive days; (3) a large number of patients in order to assure an even distribution of the many prognostic factors between the treated and the control group; (4) the administration of the most adequate combination of antibiotics to all the patients (transfused and controls); and (5) the use of strict criteria for evaluation: infectious morbidity being often difficult to interpret, the incidence of death by infection is mandatory.

The hazards and inconveniences of antibiotics are probably less than those of PMN collection and transfusion. Therefore, antibiotics alone would be preferred in the event that studies fulfilling the requirements indicated above would fail to demonstrate a higher percentage of survivors in the transfused group.

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NON-BACTERIAL INFECTIONS ASSOCIATED WITH NEOPLASTIC DISEASE

D. Armstrong, H. Chmel, C. Singer, M. Tapper and P. P. Rosen

Departments of Medicine (Infectious Disease Service) and Pathology, Memorial Sloan–Kettering Cancer Center, and Cornell University Medical College, New York, New York, U.S.A.

There are a large number of non-bacterial microorganisms waiting to infect the host with neoplastic disease. The basic disease may make the individual more susceptible, but more often therapeutic measures are responsible. Although a specific neoplastic disease may predominantly alter one immunological function, anti-neoplastic chemotherapy regimens usually alter to some degree, the others. In the appropriate setting, the clinical picture due to a specific microorganism is frequently recognizable, or at least limited to a selected group of organisms. Diagnostic measures should be prompt and specific, and treatment instituted early, for these infections are frequently life-threatening in the immunosuppressed host. Candida species have been the most common non-bacterial organisms found at post-mortem at Memorial Sloan–Kettering Cancer Center, followed by Aspergillus and Mucor species. Pneumocystis carinii infections were seen frequently during the time period observed in the study. Life-threatening infections with other non-bacterial agents were less frequent, but occurred, and frequently more than one microorganism was associated with the death of the patient.

INTRODUCTION

FUNGAL, parasitic and viral infections in the immunosuppressed host are becoming more common, [1–4] probably due to more intensive immunosuppressive therapy and more effective control of previously lethal bacterial infections. The diagnosis of non-bacterial infection is frequently difficult because the organisms (1) may be difficult to isolate, (2) are so common that isolation is of questionable significance, or (3) invasive infections can only be diagnosed serologically so that in an acute illness interpretation may depend on an acute serum alone. Even when the diagnosis is made, there may be no treatment or only regimens that employ highly toxic agents. This paper will attempt to evaluate (1) the source of the infections, (2) the clinical setting in which they may be expected to occur, (3) the diagnosis and (4) the treatment. In addition, results of autopsy protocols for the first six months of 1973 have been reviewed in an attempt to estimate the relative significance of non-bacterial infections at Memorial Sloan–Kettering Cancer Center during this time.

MATERIALS AND METHOD

In instances where large series from other institutions were available, these were compared with the most recent reviews from Memorial Sloan–Kettering Cancer Center. Our own approach to the various infections were then outlined.

Autopsy protocols for the first 6 months of 1973 were reviewed and significant infections contributing to death according to the prosecutor's description were recorded. Slides were not reviewed.

PREDISPOSING FACTORS

The predisposing factors and pathogenesis of infection in patients with an underlying neoplastic disease vary with the disease, the chemotherapy given, the type of organism infecting and the degree of exposure to the organisms [5, 6]. Table 1 contains an outline of some of the factors influencing the outcome of these host–parasite relationships.

It cannot be overstressed that antineoplastic chemotherapy can render patients deficient in all known parameters of immune defenses. Thus, a patient's underlying disease may effect one or more factors and his chemotherapy may effect the rest. In addition, the hospital environment and antibiotic therapy increase the numbers of certain organisms such as the Candida species allowing them to invade more readily.

Successful treatment of one of these infections does not change the underlying immune defects which make the host susceptible. Thus the patient may soon contract another infection with similar or even the same organism. At times simultaneous dual infections occur.

Table 1. Factors influencing exposure to and infection with selected organisms

Organism	Environmental factors External	Environmental factors Internal	Host factors Integument	Neutrophile	Monocyte	Humoral
Aspergillus spp.	dust?	altered normal flora?	±	+++	?	?
Mucor spp.	?	altered normal flora?	±	+++	?	?
Candida spp.	IV lines foley catheters	altered normal flora by antibiotics	+++	++	++	+?
Cryptococcus neoformans	Pigeon feces soil	?	–	–	+++	+?
Histoplasma capsulatum	River Valleys (especially Mississippi) in USA	?	–	–	+++	+?
Coccidioides immitis	S.W. North America Central America N. South America	?	–	–	+++	+?
Pneumocystis	Person to person?	Primary reservoir latent infection?	–	–?	+++	+++
Toxoplasma	Raw meat, cats Blood transfusion	Primary reservoir latent infection?	–	–	+++	++
Strongyloides	Fecal-contaminated soil	Primary reservoir latent infection?	–	–	+++	?
Malaria	Endemic areas, Blood transfusions	?	–	–	+++	+++
Herpes simplex	Person to person	latent infection	–	–	+++	++?
Varicella-zoster	Person to person	latent infection	–	–	+++	++?
Cytomegalovirus	Person to person Blood transfusion	latent infection	–	–	+++	++?
Vaccinia	Iatrogenic Person to person	–	+++	–	+++	++?
Measles	Person to person	–	–	–	?	++?

FUNGI

Aspergillosis species

Source: Some Aspergillus species, most commonly *Aspergillus fumigatus*, are found in dust and dirt and occasionally in the upper respiratory tract of normal asymptomatic individuals. People with asthma, bronchiectasis, or old cavitary lung disease may carry the organism in the lower respiratory tract, frequently without symptoms; thus, the reservoir may be in both the external and internal environment.

Clinical settings. Progressive, invasive disease due to Aspergillus species occurs normally in the heavily immunosuppressed host with leukopenia receiving broad spectrum antibiotic therapy. Table 2 lists the underlying diseases in patients from 2 recent large series [1, 7]. This fungus, along with *Pseudomonas aeruginosa* and mucor, has a propensity to invade blood vessels, causing thrombosis with infarction and hemorrhage [8]. There has been a definite increase in prevalence over the past 10 years at Memorial Sloan–Kettering Cancer Center, and the clinical presentation frequently has been similar. A patient with acute leukemia, under intensive chemotherapy, is leukopenic and receiving broad spectrum antibiotics for a documented or suspected bacterial infection, frequently with *Pseudomonas aeruginosa.* Fever recrudesces and pulmonary infiltrates appear which may be associated with pleuritic pain. The roentgenographic findings vary from a wedge-shaped peripheral infiltrate to interstitial infiltrates to a progressive "fungus ball."

Diagnosis. An *antemortem* diagnosis is difficult to make. Sputum usually does not reveal Aspergillus on smear or culture, but a potassium hydroxide wet mount is more likely to yield recognizable organisms than a gram stain. Positive sputum cultures are not diagnostic; we have isolated the organism in the appropriate clinical setting without evidence of invasive disease, so false positive cultures can occur. An immunodiffusion test using serum concentrated three times has been reliable in many instances when serial serums are available and a conversion from

Table 2. Invasive aspergillosis infections

Diagnosis	All cases MSKCC[1] [1] 93 Cases	NCI[2] [7] 98 Cases	Combined 191 Cases	%	Disseminated MSKCC 23 Cases	NCI 34 Cases	Combined 57 Cases	%
ALL[3]	26	41	67	54	5	17	22	57·8 %
AML[4]	16	21	37		4	7	11	
CML[5]	6	9	15	7·8	2	2	4	7·0 %
LSA[6]	11	5	16		2	–	–	
HD[7]	7	5	12	18	1	1	2	12·2 %
RCS[8]	7	–	–		3	–	–	
Solid tumor	15	2	17	8·9	5	1	6	10·5 %
Other[9]	5	15	20	10·4	1	6	7	12·2 %

[1] Memorial Sloan–Kettering Cancer Center
[2] National Cancer Institute
[3] Acute lymphocyte leukemia
[4] Acute myelogenous leukemia
[5] Chronic myelogenous leukemia
[6] Lymphosarcoma
[7] Hodgkin's disease
[8] Reticulum cell sarcoma
[9] No neoplasm

negative to positive precipitins is documented [9]. False negative tests have occurred, sometimes associated with hypogammaglobulinemia. Biopsy of pulmonary or skin lesions may be advisable in some cases. In the absence of satisfactory laboratory tests, a clinical diagnosis may have to suffice and appropriate therapy instituted early. Only a few survivors have been reported and this was almost always where the diagnosis was made early and the leukemia went into remission during amphotericin B therapy.

Treatment. Amphotericin B [10] is the only antifungal agent with proven effect against invasive aspergillosis. The drug can be initially administered on a daily, 12-hourly or even 6-hourly basis. It must be given intravenously and some patients will react with hypotension, bronchial constriction or cardiac arrythmias. Most patients develop shaking chills and fever and some nausea and vomiting. We therefore, administer a 1 mg test dose in 250 ml of 5 % dextrose water, and if this is tolerated, the dose is increased by 5–10 mg every 12–24 hr, depending upon the severity of the illness. On reaching 30–40 mg/day, most adults will show a rise in BUN and creatinine, the older the sooner. The dose should then be maintained or decreased so that the creatinine remains at 3·0 mg % (BUN 30 mg %) or below. Most people will not tolerate more than 0·7 mg/kg/day or 1·4 mg/kg every other day. An every-other-day schedule is preferable because it's much more comfortable for the patient, blood levels appear to be comparable, and clinical responses the same. Hypoalkemia and hypomagnesemia result from the renal damage and should be anticipated by supplementing their intake. With long term therapy, decrease in red blood cell production should be anticipated regularly, and, far less often, platelet and leukocyte levels may become depressed. Hepatotoxicity occasionally occurs. Phlebitis at the IV site is said to diminish with 100 units of heparin in each infusion, but controlled studies are lacking. Hydrocortisone, 50–100 mg with each infusion, does decrease the chills and fever.

Surgery has cured one patient with acute myelogenous leukemia and an invasive pulmonary "fungus ball" due to aspergillosis [1, 9]. This patient lived for two years without evidence of recurrence. This approach may prove useful in some cases.

Although there have been reports of cures of aspergillosis with 5-fluorocytosine, [11] the degree of invasiveness was not clear in these cases. Clotrimazole has been reported to have antifungal activity *in vitro* against most pathogenic fungi, but clinical studies have been disappointing [12, 13].

Mucormycosis

Source. The Mucoraceae (general Rhizopus, Mucor and Absidia) are widespread in nature, found in environmental dust and in the respiratory and gastrointestinal tracts of man. The source of the infection in most cases is unknown.

Clinical setting. As with aspergillosis, mucormycosis frequently complicates leukemia and lymphoma, as shown in Table 3, and disseminated mucormycosis has increased in prevalence in patients with leukemias [2, 14, 15–17]. It is important to emphasize that the classic triad of orbital cellulitis, sinusitis and diabetic acidosis is not the typical presentation in patients with leukemia and lymphoma. Although chemical diabetes was present in many of the patients in our series, none were in acidosis. The majority of patients had far advanced neoplastic disease and were on intensive antineoplastic and antibiotic therapy. Most were leukopenic,

Table 3. Mucormycosis

Diagnosis[1]	All cases MSKCC[2] 26 Cases	Palo Alto Stadford[14] 8 Cases	AFIP[2] [16] 33 Cases	BARNES[30] 6 Cases	NIH[3] [17] 6 Cases	DUKE[17] 6 Cases	Combined 85 Total	%
AML	11	1	3	1	6	3	33	38·8 %
ALL	5			1		2		
CLL[4]	4			1		1	6	7·0 %
CML			2	1			3	3·5 %
Unclassified Leukemia			2				2	2·4 %
LSA	2						10	12 %
RCS	2							
HD	1	2	1	2				
Solid Tumor			4				4	4·7 %
Other	1	5	21				27	32 %

[1] See Table 2
[2] Armed Forces Institute of Pathology
[3] National Institutes of Health
[4] Chronic lymphocytic leukemia

but this was not as prevalent as in patients with Aspergillosis. Thus, the predisposing factors are complex and incompletely resolved.

Diagnosis. The diagnosis is rarely made *antemortem.* Since our index of suspicion has been high, on two occasions, vigorous nasal scrapings have revealed the organism on wet mount and culture. Gram stain has not been helpful. We have not recovered it from the sputum. Biopsy of skin, palatal or pulmonary lesions may yield an *antemortem* diagnosis. Fever and pulmonary infiltrates, with no other obvious microbial cause, in the appropriate clinical setting, should suggest mucormycosis as well as aspergillosis. Less often, the rhinocerebral form presents with black, necrotic nasopharyngeal lesions, opthalmoplegia, proptosis and coma. Since mucormycosis, aspergillosis, or *Pseudomonas aeruginosa* can cause similar facial and pulmonary lesions, after appropriate studies have been obtained, treatment with amphotericin B, gentamicin and carbenicillin should be instituted. It should be noted that Mucor species have been responsible for progressive "fungus balls" as well as aspergillosis, while *Pseudomonas aeruginosa* has not.

Treatment. Amphotericin B should be administered on a schedule to reach therapeutic levels as quickly as possible. Surgery has been used to remove infected and infarcted areas successfully. Early therapy is imperative; recoveries are rare. Proven preventative measures have not been established.

Candidiasis

Source. Candida species and *Torulopsis glabrata* are widespread in nature and found as normal flora in cultures of the gastrointestinal and gynecological tract of man. Antibiotic therapy depresses normal bacterial flora and encourages Candida overgrowth. The higher the dose and broader the spectrum, the more Candida flourishes. Candida also likes moist areas, such as the perineum, and those with a high glucose content, such as the urine of a diabetic.

Clinical setting. Diabetics, children, patients on corticosteroids and women on contraceptive pills are more susceptible to Candida than the general population, as are patients with leukemia and lymphoma, especially when they are under therapy with cytotoxic agents, corticosteroids and antibiotics [14–19]. Whether neutropenia adds to this is uncertain, for most neutropenic patients are at least on cytotoxic agents and antibiotics. Patients with stomatitis due to chemotherapy or secondary to *Herpes simplex* are especially prone to Candida superinfection. In patients with leukemia and lymphoma with the above added predispositions, oral infections may be followed by esophagitis or invasive infection along the lower gastrointestinal tract with ulceration, bleeding, perforation or dissemination. Rarely, a primary pneumonia occurs in this type of patient. Urinary tract infections may occur, especially in the presence of a Foley catheter, and dissemination may result from renal infection. This type of infection accounts for many of the cases of candidiasis seen in patients with solid tumors. Patients who have had bladder or gynecological surgery, particularly those resulting in ileal bladders, are prone to urinary tract infection. In this setting, Candida may grow so exuberantly in the urinary tract that obstruction due to mycelial mats or "fungus balls" occurs either at the uretero-vesicle, uretero-pelvic junctions or in the tubules.

Intravenous indwelling catheters or even needles are prone to superinfection in hospitalized patients.

Thus, all patients, whether they have solid tumors or leukemias and lymphomas, are at risk, especially if their normal flora has been overgrown with Candida due to antibiotic therapy.

Bacterial abscesses or fistulas, after extensive abdominal surgery and successful treatment with antibiotics, may become superinfected with Candida.

The situations outlined above are reflected in the underlying neoplastic diseases listed in Table 4 [14].

Table 4. Candidiasis

Diagnosis[1]	All [14] Palo Alto/Stanford 42 Cases	Case [20] MSKCC 101 Cases	Case [20] Combined 143 Total	%
Solid tumor	10	58	68	47·2 %
Lymphoma	5	13	18	12·5 %
Acute leukemia	2	14	16	11·1 %
Chronic leukemia		2	2	1·4 %
Other	25	14	39	27·2 %

[1] See Table 2 for abbreviations.

Diagnosis. The diagnosis of invasive Candidiasis is one of the most difficult in infectious diseases [5]. The organism is extremely prevalent in hospitalized patients on anti-neoplastic and antimicrobial therapy and its isolation represents far more often colonization rather than invasive infection. Pseudo hyphae, once thought to represent invasive infection, may be present in colonization. They may be present in bronchial washings when the bronchoscope is passed through the mouth. Even the isolation of Candida from a blood culture may only represent a transient fungemia from a catheter which does not recur after removal, or from an undocumented site which does not progress.

Serological tests for invasive Candidiasis have been varyingly successful [21–23]. We have found both false positive and false negative results using an immunodiffusion and agglutination test on acute and convalescent specimens [23].

Because smears, cultures and serological tests are of limited usefulness, the clinician must weigh all the factors for invasive infection with Candida against other microorganisms present in cultures and other serological tests and make a clinical judgement as a result. The ideal diagnostic procedure is a biopsy of the organ suspected of infection, such as lung biopsy.

Treatment. The first step in treatment is to discontinue all predisposing factors when possible. Corticosteroids should be tapered, antibiotics stopped, catheters removed, etc. For local infections, nystatin or miconazole solutions or ointments can be used. In oral Candida infections in the immunosuppressed host, we treat early and vigorously with nystatin mouth wash, "swish and swallow" 10 million units g.i.d. plus nystatin suppositories given by mouth as a lozenge (to melt in the mouth) two to four times a day. There is obviously a need for a lozenge made for that purpose. We feel that this clears up the local lesions more effectively than any other regimen, but have no evidence that it prevents esophagitis or gastrointestinal lesions. In the face of mild symptoms of esophagitis, we use the nystatin regimen outlined above, but if symptoms are severe and associated with otherwise unexplained systemic signs, we treat with amphotericin B. Doses between 20 and 30 mg/day are usually effective, and a 10 day to 2 week course necessary. Although pneumonias are rare, we have found it necessary in one biopsy-proven case to use higher doses of amphotericin B – up to 60 mg/day in a 40 kg patient. After 6 weeks of therapy, there was no recurrence. In heavily infected lower urinary tracts, before urinary shutdown may supervene in the "fungus ball" type of syndrome, we first try local instillation of amphotericin B, 50 mg in 1 l. of sterile water twice daily. If this is not effective, systemic therapy with 20 to 30 mg of amphotericin B per day is usually highly effective, resulting in increased urine output and improved renal function. Two weeks of therapy is usually sufficient. With renal infections, higher doses and longer therapy are usually required, but it varies from patient to patient.

Indwelling i.v. catheters present a special problem. Removal of the infected catheter may be all that is necessary. There is no valid way of determining this and early treatment with amphotericin B may be beneficial and prevent infection of a new catheter if that is necessary. We remove the catheter, treat with amphotericin B if otherwise unexplained systemic signs are present, and try to wait until

effective blood levels are present before a catheter is reinserted. The duration of treatment will vary according to whether the organisms have infected the kidneys, lungs or other organs.

For wound infections, usually low dose, short duration therapy with adequate drainage is sufficient.

Even with a high index of suspicion, most of our disseminated Candidiasis is found at autopsy.

Cryptococcosis

Source. Cryptococcus neoformans is widespread in soil and man and other mammals are frequently infected. Disease in man is rare, and about one-half the reported cases have occurred in individuals with an underlying disease [14, 24, 25]. Soil appears to be the source, especially that containing pigeon feces, and mammal-to-mammal spread has not been documented, although pets such as dogs and cats or domestic animals such as cows do become infected.

Clinical setting. Almost one-half of our patients with neoplastic disease infected with *Cryptococcus neoformans* have had highly advanced Hodgkin's disease and were on one or more chemotherapeutic agents, particularly corticosteroids [25]. Other lymphomas and leukemias comprised the underlying disease in almost all the rest (Table 5). Similarly, they were far advanced and intensively treated. In a patient with leukemia or lymphoma under intense chemotherapy who presents with neurologic symptoms of insidious onset, a search for cryptococci should be made.

Diagnosis. The same type of patient develops infections with *Listeria monocytogenes,* the organism most likely to imitate CNS infection with Cryptococcus and Toxoplasma or Nocardia, which are more often present as brain abscess or encephalitis [26, 27]. The diagnosis of *Cryptococcus neoformans* central nervous system infection depends upon isolating the organism from cerebrospinal fluid (CSF), blood, urine or other body fluids or from biopsy specimens such as skin and lung. Cryptococcal antigen has been detected in the absence of culturable organisms and this has been advocated as an indication for treatment [28]. We have never had to do this, for whenever we have detected antigen, if the organisms were not present in the CSF on that specimen, we have been able to obtain sufficient CSF on a subsequent attempt to isolate the organism. It is highly desirable to do so, since not all isolates are sensitive to 5-fluorocytosine, and it helps to know this before embarking on therapy with a potentially toxic drug [11, 29]. When spinal taps are positive for antigen and negative on smear and culture, if 10 ml of fluid does not yield the organism, then even more fluid or a ventricular puncture collecting 50 ml of CSF has been successful.

Treatment. The only reliable method of treating *Cryptococcus neoformans* infection is with amphotericin B, using the schedule outlined under aspergillosis. Cerebrospinal fluid levels of amphotericin B are frequently barely detectable; however, a clinical response of the meningitis is the rule. If intrathecal therapy is felt necessary due to the fulminant nature of the infection, lack of response to IV therapy or

Table 5. Cryptococcosis

	All Cases				
Diagnosis	AFIP[2] [24] 60 Cases	Palo Alto Stanford[14] 9 Cases	MSKCC[25] 46 Cases	Combined 115 Total	%
HD	11	2	19	32	
LSA			9	9	42·6 %
RCS	2		5	7	
Lymphoma		1		1	
AML		1		1	1·7 %
ALL			1	1	
CLL		3		3	4·3 %
CML			2	2	
Unclassified leukemia	4			4	3·4 %
Carcinoma	1		3	4	3·4 %
Other	42	2	7	51	44·3 %

See Table 2 for abbreviations.
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relapse, then an Ommaya reservoir allowing intraventricular therapy should be inserted. Treatment should proceed cautiously with 0·01 mg intraventricularly in 5 ml of 5 % dextrose water and 5 ml CSF. Every other day thereafter, 0·03, 0·05, 0·08, 0·10, and so on can be given until doses of 0·5 mg, two to three times a week are reached. CSF from lumbar punctures as well as ventricular fluid should be followed for response, for the ventricular fluid may demonstrate a pleocytosis and elevated protein as a result of the therapy alone. In systemic cryptococcosis, i.v. therapy should be continued along with intraventricular, although it will usually be necessary to continue the latter longer. Cryptococcal antigen in the CSF and blood should be followed. With the return of the CSF parameters to normal and the disappearance of antigen, therapy should be continued for a month or two longer and then stopped with continued monitoring by examination of CSF and antigen in blood and CSF on a monthly basis.

Five fluorocytosine (5FC) has been used in the treatment of cryptococcosis, but since some isolates are resistant *de novo*, and many develop resistance during therapy, this drug should not be used alone [11, 29]. In conjunction with amphotericin B, 5-fluorocytosine may enhance therapeutic effects, but this remains to be proven by controlled studies.

Histoplasmosis:

Source. Histoplasma capsulatum is widespread in soil and the excreta of various animals. Asymptomatic infections predominate in the general population. Outbreaks have been traced to inhalation of spores from contaminated soil from starlings or blackbird roosts, from bat guano and from chicken manure.

Clinical setting. Disseminated Histoplasmosis has been described in patients with leukemias and lymphomas more than in solid tumors [15, 30–33] (Table 6).

Diagnosis. The diagnosis rests on isolation of the organism or a four-fold rise in antibody titer. Therapy should be started, however, if the organism is seen on bone marrow smear or biopsy. Bone marrow aspiration and culture, as well as blood culture will yield the diagnosis in about 10 % of patients. Elevated (>1:8) complement fixation titers or the presence of Y bands on immunodiffusion tests suggest active disease.

Treatment. Amphotericin B is the treatment of choice, as outlined under aspergillosis.

Other fungi:

Blastomycosis, sporotrichosis and coccidioidomycosis do not seem to have a predilection for patients with neoplastic disease. Coccidioidomycosis has, in individual cases, been particularly severe in immunosuppressed hosts.

PARASITES

Pneumocystis carinii

Source. The source of *Pneumocystis carinii* infections may be from an endogenous latent focus or by person-to-person spread. Most cases seem to be from the former, but there are suggestive cases of the latter [34, 36] and we have recently had a cluster of cases which appeared to be the result of person-to-person spread or from a focus in our hospital [37]. How often this occurs is unknown; we do place our patients with suspected *P. carinii* pneumonia on respiratory precautions. It will be difficult to estimate the source until methods for isolating the organism *in vitro* and/or better serological methods become available.

Table 6. Histoplasmosis

Diagnosis[1]	Barnes[30] 7 Cases	NCI[2] [15] 5 Cases	ST. Judes[31] 6 Cases	CDC[3] [32] 54 Cases	NIH[4] [33] 26 Cases	Combined 98 Cases	%
ALL			6			6	13·2 %
AML		5			2	7	
CML	1			1		2	3·0 %
CLL					1	1	
HD	1			1	1	3	3·0 %
Solid tumor				1		1	1·0 %
Other	5			51	22	78	79·5 %

[1] See Table 2 for abbreviations.
[2] National Cancer Institute.
[3] Center for Disease Control.
[4] National Institutes of Health.

Clinical setting. P. carinii takes advantage of both monocyte defects and hypogammaglobulinemia [38, 39]. Table 7 lists the underlying diseases from a number of series. In patients with neoplastic disease, those with Hodgkin's disease or acute lymphoblastic leukemia are the most susceptible. Hodgkin's disease is usually far advanced and has been intensively treated when *P. carinii* pneumonia occurs [37–40]. In contrast, many cases in acute lymphoblastic leukemia occur in patients in remission [37, 41]. Pneumonias have appeared often enough during tapering of corticosteroid therapy to suggest some association [37, 42] and to raise the question of a component of immunological reaction in the pathogenesis of the disease. The clinical presentation has been that of an interstitial pneumonitis accompanied by dry cough, dyspnea and fever and only rarely has *P. carinii* been found outside of the lungs [38].

examined, bleeders carefully tied-off, and a tube left in the chest to control pneumothorax in patients whose pulmonary reserve is precarious [43]. Since there are so many other possible causes of an interstitial pneumonitis in the patients who might develop pneumocystis [43, 44], we feel that it is absolutely necessary to make a specific diagnosis since treatment to cover all possibilities is toxic, and time is lost by empirical trials.

An indirect immunofluorescent test for *P. carinii* antibody has been developed, but the test is positive in only about one-third of proven cases [45]. Rises or falls in titers have been demonstrated, but many patients with active infection have no detectable antibody and normal individuals on a single serum may have titers as high as infected patients [37]. Other serological methods are still under study.

Table 7. Pneumocystis carinii pneumonia

Underlying disease[1]	CDC[2] 1974[39]	U. Minnesota Hosp. 1973[38]	MSKCC 1972[40]	St. Judes 1973[41]
Primary immune deficiency	25(12·9 %)	15(32·6 %)	0	0
Leukemia Total	91(46·9 %)	11(24 %)	4(20 %)	45(88 %)
ALL	58	3	2	41
CLL	18	1		
AML	9	2		4
CML	3	4	2	
Other leukemia	3	1		
Hodgkin's disease	21(10·8 %)	2(3·9 %)	6(30 %)	1(2 %)
Other lymphomas	13(6·7 %)	7(15·2 %)	6(30 %)	1(2 %)
Solid tumors	7(3·6 %)	3(7·4 %)	3(15 %)	3(5·9 %)
Organ transplants	22(11·4 %)	3(7·4 %)		
Collagen disease	9(4·6 %)	1(2 %)		
Miscellaneous	6(3·1 %)	4(8·7 %)	1(5 %)	1(2 %)
Total	194	46	20	51

[1] See Table 2 for abbreviations.
[2] Center for Disease Control.

Diagnosis. Demonstration of the organism by histopathology is the only reliable method of diagnosis. It has not been grown *in vitro.* It has been seen by staining methods in sputum, tracheal aspirates, bronchial washings or brushings and needle and open lung biopsies.

Initially, we prefer to study sputum specimens or bronchial washings and if these are negative, move rapidly to open lung biopsy. Many of our patients are thrombocytopenic and the tendency to bleed requires caution in any "blind" procedure, including nasotracheal or transtracheal aspirates, bronchial brushings or needle aspirations. With an open lung biopsy, the patient's breathing is controlled by an endotracheal tube, the tissue for biopsy can be

Treatment: Pentamidine 4 mg/kg/day i.m. is the only well-studied treatment for *P. carinii* pneumonia, resulting in about a 50 % cure rate [39]. The recommended course is 10–14 days; we have treated longer in patients who responded slowly. Adverse reactions include sterile abscesses at the site of the inoculation, azotemia, hypoglycemia, and, on i.v. administration particularly, hypotension [39]. The latter route is not ordinarily recommended, but we have used it with success in severely thrombocytopenic patients.

Sulfonamides with pyrimethamine, in doses similar to those used for toxoplasmosis have been used in a limited number of cases, [46] but a large enough series to evaluate its efficacy has not been done.

Animal experiments suggest that sulfamethoxazole-trimethoprim may be better than pentamidine in the treatment of *P. carinii* pneumonia [47].

Toxoplasmosis

Source. The known sources of toxoplasma infections for man are (1) uncooked meat, (2) cat feces, (3) congenital toxoplasmosis which may well be the source of endogenous, latent infections which disseminate or become invasive with immunosuppression, (4) transfusions have been implicated recently and (5) accidental inoculation by laboratory personnel. Whether other, undocumented sources exist remains to be proven [48].

Clinical setting. The underlying neoplastic diseases in patients with invasive toxoplasmosis as reported in a number of series are listed in Table 8. It is most prevalent in lymphomas, especially Hodgkin's disease, and leukemias. Most patients were on chemotherapy. Fever with or without lymphadenopathy or rash may be the only signs, but central nervous system signs of diffuse encephalitis or focal lesions occur in more than 1/3 of cases from the two largest series [49, 50]. The same type of patient that develops toxoplasma CNS disease may well develop similar signs and symptoms from *Nocardia asteroides, Mycobacterium tuberculosis,* or the fungi mentioned above. The pulmonary signs of toxoplasmosis are indistinguishable from any number of agents in the same clinical setting. Toxoplasma pneumonitis is frequently found in combination with cytomegalovirus. Finally, the underlying neoplastic disease or the results of chemotherapy can mimic the signs and symptoms of toxoplasmosis, including myocarditis.

Diagnosis. The organism has been isolated from lymph node and brain biopsy and if tissue is available, mouse inoculation can be valuable. Histopathology usually reveals the organism in brain or lung tissue, but less likely in lymph nodes. A typical lymphadenitis is seen. In the absence of identification of the organism by isolation or histopathology, a serological diagnosis must be employed. In an acute illness, the clinician may not be able to wait for a 4 fold titer rise. Different tests and titers suggesting acute disease for the various types of serology are indicated in Table 9. It should be stressed that these are not diagnostic. Even IgM titers by indirect immunofluorescence may remain elevated for months after an acute infection, or cases have been followed where preexisting antibodies have fallen as the toxoplasmosis became more severe [50, 51].

Treatment. A sulfonamide, preferably sulfadiazine, should be administered at 6 to 8 g per day with particular attention to hydration to prevent precipitation of crystals in the kidney tubules. Pyrimethamine in doses of 50–75 mg/day should be given along with folinic acid, 6 mg/day to avoid marrow toxicity [52]. In CNS disease, pyrimethamine does cross the blood brain barrier, but in one cured case it was administered by an Omaya reservoir [49].

Experimental work in mice suggesting that clindamycin may be effective should be studied in humans. The role of surgery in toxoplasmosis brain abscesses also remains to be proven.

Strongyloides

Source. The source of strongyloides is soil

Table 8. Underlying neoplastic diseases in invasive toxoplasma infections

	MSKCC[50]	Others[48, 52]	Combined	Percentage
Hodgkin's disease	9	12	21	39·6 %
Lymphogranuloma		4	4	7·5 %
Histiocytic lymphoma		1	1	1·9 %
Lymphosarcoma	1	3	4	7·5 %
Multiple myeloma	1		1	1·9 %
Chronic lymphocytic leukemia	1	4	5	9·4 %
Myeloid metaplasia	1		1	1·9 %
Chronic myelogenous leukemia	1	3	4	7·5 %
Acute myelogenous leukemia		2	2	3·8 %
Acute lymphocytic leukemia		4	4	7·5 %
Solid tumor	1	5	6	11·3 %
			53	

Table 9. Toxoplasma serology

Test	Titers suggesting* recent infection
Sabin-Feldman dye test (SFDT)	≥1:1024
Complement-fixation test (CFT)	≥1:8
Hemagglutination test (HA)	≥1:1024
Immunodiffusion test (ID)	+ band(s)
Indirect immunofluorescent test (IFA)	≥1:1024
Total serum immunoglobulins Igs	
immunoglobulin G IgG	≥1:1024
immunoglobulin M IgM	≥1:256

* A four-fold rise in titer is diagnostic of ongoing infection between the serums tested. Conversion from a negative to positive ID test is strongly suggestive.

contaminated with human feces or the patient's own g.i. tract where the infection has been latent.

Clinical setting. In the dozen or so cases of disseminated strongyloidiosis or "hyperinfection syndrome," the majority have been in patients with lymphomas or leukemias and all have been on prednisone [53–59]. All were from endemic areas. Patients usually showed signs of decreased intestinal motility.

Diagnosis. A high index of suspicion for any patient from an endemic area and stools for ova and parasites before immunosuppression are mandatory. During dissemination eosinophilia may disappear but organisms may be seen in the feces or sputum. Duodenal aspiration may be necessary to make the diagnosis and should be done immediately when it is suspected.

Treatment. Steroids should be decreased and thiobendazole administered early at doses of 1 g/day for 2 days to start. Up to 4 g have been used in some cases. Levamisol has been recommended on the basis of animal studies and is worth a trial for its immune aduvant effect as well as anti-nematode activity. Most cases have died with or without treatment.

Giardiasis

Infection with the protozoan parasite *Giardia lamblia* has been reported in association with a variety of immunodeficiency states. The majority of those cases has been reported in association with "acquired dysgammaglobulinemia" in conjunction with a lowered serum IgA level [60–62]. There are additional cases of giardiasis reported in a patient with congenital x-linked agammaglobulinemia [63] and in a patient with Hodgkin's disease with hypogammaglobulinemia [64]. Frequently, the parasite is not identified even on repeated stool examination, and jejunal biopsy may be necessary to document the infection. Reversible abnormalities in intestinal mucosal and villous architecture are commonly seen in this group of patients with return to normal histology and absorptive function after treatment [65]. Metronidasole 250 mg by mouth 3 times daily for 10 days is the therapy of choice.

Malaria

Considerable experimental evidence in animals and a growing list of cases in man support the conclusion that splenectomy is associated with a more fulminant infection with malaria. Latent malaria may be reactivated in a previously asymptomatic individual by splenectomy and even the benign forms of malaria (vivax or malariae) may follow a more malignant course in a splenectomized host [66, 67]. The role of immunosuppressive chemotherapy in the course of malaria is less clear – some authors have reported that latent malaria may be reactivated in patients undergoing immunosuppression, but the number of cases is too few to allow any general conclusions [68].

Ameba

A single case has been reported of a patient with Hodgkin's disease who was found at *postmortem* examination to have multiple brain abscesses and a mild meningitis ascribed to a Hartmannella sp. [69]. Unlike most previously reported cases of amebic meningoencephalitis, there was no mention of a history of swimming or diving in this patient's background.

VIRUSES

Herpes simplex

Source. Herpes simplex commonly causes a latent infection in the oral cavity called *Herpes*

labialis (*Herpes simplex* type I.), or in the genital area (*Herpes simplex* type II.) called *Herpes progenitalis* [70]. Recurrent latent oral infections are usually the type seen in patients on intensive chemotherapy, especially for leukemias and lymphomas. New infections with either Type I or II have not been documented to our knowledge in patients with neoplastic disease, and in the rare case of dissemination of a *Herpes simplex* in a patient with leukemia or lymphoma, the organism has not been typed.

Thus, the source of severe *Herpes simplex* infections in patients with neoplastic disease appears usually to be latent oral infection.

Clinical setting. A few patients with far-advanced lymphomas have developed hematogenously disseminated *Herpes simplex* including skin lesions resembling varicella. Other patients with lymphomas and leukemias have shown visceral involvement with inclusions suggesting *Herpes simplex* [71]. During 1971 and 1972 we saw four cases of invasive *Herpes simplex* observed at autopsy, 2 in patients with Hodgkin's disease, and 1 each in patients with ALL and RCS [3]. In three of these, the infection was of the respiratory and upper g.i. tract. Only one had histologic evidence of hematogenous spread to liver and pancreas. All four patients had evidence of multiple other opportunistic infections.

Diagnosis. The only definitive means of diagnosis is to isolate and identify the organism. It helps if inclusions are seen on smears of mouth lesions, since *Herpes simplex* is sometimes isolated from the saliva of people without lesions. Serological responses are irregular in recrudescent *Herpes simplex.* In severe mouth infections, the vesicular stage may pass quickly so that ulcers are the first lesions noticed.

Treatment. There is no proven effective treatment for Herpes simplex infection other than iritis, where the conditions are suitable for effective, local idoxyuridine [72] application.

Varicella zoster.

Source. Varicella (chicken pox), in an unknown percentage of cases, results in a latent infection in the host – at unknown sites in the body, possibly in dorsal root ganglia of the spinal and cranial nerves. Subsequently, for unknown reasons, but more frequently in immunosuppressed hosts, activation of the latent infection occurs, manifested by the appearance of varicella-zoster (V-Z) virus-induced vesicles along the root or roots of a sensory nerve (*Herpes zoster* or "shingles"). In some cases, this results in dissemination of the virus with few-to-many vesicles occurring by hematogenous spread. More rarely, a varicella pneumonia or encephalitis occurs.

Thus, an immunosuppressed patient who has never had varicella will usually respond to first contact with the virus by a case of varicella, which may be life-threatening. If the immunosuppressed host develops *Herpes zoster* from a latent infection, this may remain local or disseminate. The incidence of dissemination in immunosuppressed patients has been estimated to be between 15 and 32 % in different studies [73–75]. The natural history of this dissemination varies from a mild to a life-threatening disease.

Clinical setting. Hodgkin's disease is the neoplasm most frequently complicated by *Herpes zoster* [73, 74]. Other lymphomas and leukemias predispose to *Herpes zoster* more than solid tumors. A common denominator is cytotoxic and corticosteroid therapy, but lesions may appear in previously irradiated areas [74, 75] and dissemination is associated with far-advanced neoplastic disease and anergy. Varicella appearing in the previously unexposed host is most severe in children with acute lymphoblastic leukemia.

Diagnosis. Varicella is usually a simple clinical diagnosis based on a maculopapular to a vesicular eruption appearing in different stages in any one area. The rash may be atypical in immunosuppressed patients, particularly those with thrombocytopenia and hemorrhage into the lesion. If there is any real suspicion of variola or vaccinia, electron microscopy should differentiate the V-Z virus from the pox viruses on biopsy of a lesion. In almost the same amount of time it may take to arrange for electron microscopy to be done, pox viruses will grow on eggs while V-Z virus will not.

Herpes zoster may occasionally be confused with a crop of *Herpes simplex* lesions. Aspirates, scrapings or swabs of the latter will yield a typical cytopathic effect (CPE), frequently in 24 hr on human embryonic lung (HEL) and rabbit kidney (RK) cell cultures, while V-Z takes four or more days, does not grow in RK cells, and also has its own pathognomonic CPE in HEL cells. In our experience, scraping and staining smears have not been helpful in differentiating V-Z inclusions from *Herpes simplex.*

Treatment. Zoster-immune-globulin (ZIG) or zoster-immune-plasma (ZIP) are presently under study in both Varicella and *Herpes zoster* in the immunosuppressed host. ZIG definitely ameliorates

Varicella when given during the incubation period to susceptible hosts [76]. Whether ZIG or ZIP will alter the course of *Herpes zoster* and its dissemination will require a carefully controlled study with many patients, for the natural history of the disease varies, and many patients develop *Herpes zoster* in the face of endogenous antibody [74].

Since dissemination of V-Z appears to be related to delayed appearance of interferon in vesicles, human interferon, produced in lymphocyte cultures, has been used in uncontrolled pilot studies in disseminated zoster infections [77]. Its efficacy is uncertain, and controlled studies are in progress.

Chemotherapy of disseminated *Herpes zoster* with cytosine arabinoside at 100 mg/m^2/day was evaluated in a double blind controlled study and was found harmful rather than beneficial [78]. Further studies with different doses or agents will have to be as carefully controlled, and anecdotal reports detract from rather than add to the literature.

Cytomegalovirus

Source. In most cases of cytomegalovirus (CMV), the source is uncertain. In congenital and post-transfusion infections, the source is evident. The virus is obviously passed from person to person by saliva and urine and sexual contact, but when an individual develops a disseminated or invasive CMV infection, an endogenous, latent source may be responsible. Even in patients who have been followed prospectively, excretion of the virus may not be consistent and antibody levels may be too low to measure by the usual complement fixation test. Studies have shown that some cases are due to exacerbations of latent infections, but the exact incidence is and will be difficult to determine.

Clinical setting. Many patients with extensive neoplastic disease and on intensive immunosuppressive therapy carry cytomegalovirus without any evidence of invasive disease. The underlying diseases seen in two series from this institution are summarized in Table 10. In our experience in patients coming to *postmortem* with histologic evidence of widespread CMV infection, those with Hodgkin's disease have led all the rest, comprising more than 70 % of a recent series [3]. In addition, the prevalence had increased more than 4 fold in the years 1971 and 1972 over previous years. Thus, of the many patients who are excreting CMV or who have antibody, those with a lymphoma (particularly Hodgkin's disease) or a leukemia are most likely to suffer an invasive infection, and the infection is most likely to be manifest by a pneumonia or g.i. invasion with bleeding. Many patients have simultaneous invasive infections with organisms other than CMV, such as Candida, Aspergillus, *Herpes simplex*, Pneumocystis carinii, Toxoplasma, Nocardia, and various bacteria.

Diagnosis. Isolation of the virus from body fluids does not necessarily indicate pathogenic invasion by CMV. We have isolated CMV from lung biopsies where there was no histopathological evidence of the disease. A rising antibody titer may be seen in the absence of clinical disease. Many of our patients who died with extensive CMV infections had no antibody, or antibody levels fell terminally.

Histopathologic evidence of cytopathic disease along with virus isolation would appear to be the only definitive method of diagnosis. Virus isolation or rises in antibody titer are only adjuncts to a clinical evalua-

*Table 10. Cytomegalovirus infections at autopsy – MSKCC**

Underlying disease	Rosen & Hajdu [71] 1957–1968		Armstrong & Rosen [3] 1971–1972	
Leukemia				
AML	1	(5·2 %)		
CML	2	(10·5 %)		
ALL	0			
CLL	2	(10·5 %)		
Hodgkin's disease	6	(32 %)	10	(71 %)
Lymphosarcoma	2	(10·5 %)		
Reticulum cell sarcoma	2	(10·5 %)	2	(14 %)
Solid tumors	4	(21 %)	1	(7 %)
Liver transplant			1	(7 %)
Total	19		14	

*Memorial Sloan–Kettering Cancer Center.

tion which must include a search for other likely pathogens having a predilection for that type of patient.

Treatment. There is no treatment proven effective for CMV infection.

Vaccinia and measles viruses

Source. Vaccinia has two sources – one, the well-meaning, but ignorant doctor who vaccinates the patient; and, two, medical personnel, family members or friends who have been vaccinated themselves and are shedding virus.

Measles spreads by person to person contact or by use of the live-attenuated measles vaccine.

Clinical setting. Vaccinia may infect severely any patient with leukemia or hypogammaglobulinemia for any reason. Measles, with giant cell pneumonia, has been seen in children with acute leukemia. It has been virtually unheard-of since widespread, early use of the measles vaccine in the United States.

Diagnosis. Vaccinia is diagnosed by smear and electronmicroscopy of a lesion, or by culture. Measles is a clinical diagnosis, for the isolation and identification or rise in antibody titer is usually not attained early in the disease. Lung biopsy may be necessary to identify the giant cell pneumonia.

Treatment. During the incubation period of vaccinia, semithiocarbazone may be effective in preventing or ameliorating disease, as may vaccinia immune globulin.

There is no known treatment for measles virus infection.

RECENT MSKCC EXPERIENCE

During the first 6 months of 1973 there were 265 autopsies done at Memorial Center. Forty-six significant non-bacterial infections and the broad category of underlying diseases are listed in Table 11. In contrast, there were 77 significant bacterial infection in the 265 patients.

Candida species infections were the most prevalent, followed by Aspergillus and Mucor. The relatively large number of cases of *Pneumocystis carinii* pneumonia are presumably related to a cluster of such cases seen during that time [37]. The fungi were the most prevalent cause of life-threatening infections outnumbering the herpes group of viruses by 7-fold.

Table 11. Non-bacterial causes of life-threatening infections and underlying disease from autopsies for first 6 months of 1973 at Memorial Sloan–Kettering Cancer Center

	Candida	*Aspergillus sp.*	*P. carinii*	Mucor	CMV	V-2	*H. simplex*	*Toxoplasma*	*Histoplasma*	Total cases Autopsied
Hodgkin's disease	1	0	2	0	2	1	0	1	0	17
Lymphoma	3	0	1	0	0	0	1	0	0	24
Leukemia	7	5	2	5	0	0	0	0	1	33
Solid Tumor	10	3	1	0	0	0	0	0	0	185
Others	1	0	0	0	0	0	0	0	0	6
Total:	22	8	6	5	2	1	1	1	1	265

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DISCUSSION OF THE PAPERS PRESENTED

Wrigley (London)

I wonder if Dr. Tattersall would like to comment on the use of prednisone as part of the induction regimen in patients with acute leukemia. Dr. Tattersall clearly pointed out that steroids do affect the mechanisms of defense and yet it is a feature of that regime which has one of the best published results in acute myeloblastic leukemia.

Tattersall (London)

I think that the value of prednisone in the treatment of acute leukemia and in the treatment of Hodgkin's disease has been established. The value of prednisone added to other drugs in the treatment of other tumors frequently has not been established. There is a widespread feeling that predisone is a good drug to include in a combination chemotherapy schedule, but I feel that if there are real indications that predisone offers something therapeutically as an anti-tumor effect, it should be included; but there are a lot of situations where the drug is added to the protocol where the evidence for the drug's being active by itself does not exist.

Dietrich (Ulm)

Does anyone know of a controlled trial to establish the effect of predisone added to other cytostatic drugs in acute myeloblastic leukemia? I think it is an established therapy in acute lymphoblastic leukemia, but so far as I know and as I remember from the literature it is not an established therapy in acute myeloblastic leukemia.

Levine (Bethesda)

You discussed the possibility of activation of latent viruses with chemotherapy. From your studies, is there any evidence that a particular agent or a particular therapeutic schedule is more prone to activate latent viruses than another?

Tattersall (London)

The study of which I am aware and in which this proposal has been examined in some detail was relative to the high incidence of progressive multifocal leukoencephalopathy in acute leukemic patients receiving central nervous system irradiation and intrathecal methotrexate. And that is really as far as I can go. There are other drugs which in an experimental situation apparently can cause latent virus activation but in a clinical setting I don't know if there is any reason to say that one drug is more active than another.

Levine (Bethesda)

It is interesting to speculate on the possible integration of those particular viral genoms to the host nuclear material and the possibility that there are some sort of activation mechanisms which might show up their specificity with one chemotherapeutic agent, but not with another.

Phillips (London)

May I ask a question on the antibacterial activity of some anticancer drugs? First of all, are all the drugs involved in this and what is the degree of the antibacterial activity in terms of minimal inhibitory concentration (M.I.C.)? Finally, should we be taking account of it in doing blood cultures?

Tattersall (London)

The drugs which have been studied and the publication with which I am familiar looked at methotrexate and the minimal inhibitory concentrations of streptococcus. I can't remember what the relationship exactly is but I know that it is consistent with the drug level that would be obtained in man using methotrexate at a conventional dose.

The other publication which I am familiar with is the paper by Goldschmidt and Bodey who looked at 5 drugs and stated that the concentrations they were using in their experiments were consistent with drug levels obtained *in vivo* and therefore they felt that the observations they made about these drugs having a greater inhibitory effect against gram positive organisms than against Gram-negative organisms was of possible importance *in vivo*. As to the last point, should we take any notice of the presence of cytostatic drugs in the blood taken for bacteriological cultures? The answer on that is probably 'yes'. It depends on when the blood culture is taken in relationship to when the chemotherapy is given and frequently this factor is totally ignored. One should look into this and perhaps somebody is doing so. We know that some of the cytostatic drugs hang around

in the serum for a very long time; others are rapidly taken up into formed elements of the blood and others leave the circulating blood all together. I think it is something we should be aware of and something we should think about.

Schimpff (Baltimore)

I wish to make a comment about Dr. Bodey's study; the one just referred to. They studied 6 drugs and the concentrations that they referred to are probably above the concentrations that are usually found in serum. We repeated these studies and found that at levels achievable in the serum either individually or in combination we were not able to find an antimicrobial effect whether or not the drugs were used.

Chouteau (Grenoble)

We want to present our preliminary experience of disinfection in acute leukemia. The aims of our studies can be summarized in two questions: is a gut disinfection feasible as a part of the prophylaxis of infection during the induction phase of acute leukemia treatment? And does gut disinfection influence the rate of mortality resulting from infection disease?

We have found some reduction of the frequency of infection in the decontaminated patients, but our data are still in a preliminary stage.

Armstrong (New York)

Dr. Levine presented a very nice summary of the work on protected environments and gastrointestinal sterilization. What is suggested to me is that we should carefully consider discontinuing these studies. The studies cause considerable discomfort in the patients both psychologically and physically. Most of the procedures do not show a prolongation of life of the patients. We should question whether patients with acute leukemia who are already suffering while dying should be made objects of further discomfort which has little or no clinical advantage.

Levine (Bethesda)

I think that the conclusion was probably inplicit in the presentation that I gave. However at least some authors in this field would have a claim quite different from the one you stated and therefore are favorable to further studies in this field. I think however, they have to justify these biological experiments not only in terms of the cost but also in terms of the ethical aspects.

Brumfitt (London)

I should like to re-emphasize what Dr. Armstrong said. Further, I would like to suggest that the possibility of sterilizing the gut using any group of drugs at present available is negligible; the reason for this is that none of these drugs, to the best of my knowledge, has any effect on the anaerobic flora. Furthermore, the frequency of mutation of some of the bacteria in the bowel is such that you expect resistance to emerge. The end result of attempts to sterilize the gut seems to be to give more and more drugs producing more toxicity as Dr. Armstrong pointed out. The administration of oral antibiotics complicates the early recognition and treatment of infection. I will not say anything about endotoxins except that considerable doubt has been expressed about their clinical importance. I believe that data on this topic will shortly be published by Dr. Edward Kass.

Dietrich (Ulm)

I think your provocative comment is a very good one but I disagree with it. We all know there are a lot of difficulties in attempting to make a human being germ-free; the ideal situation for allowing aggressive treatment of cancer and other similar diseases. However, we have shown that in infants the realisation of a germ-free state was possible. An infant was made germ-free by antibacterial treatment and stayed germ-free for a considerable time in isolation in our unit at Ulm University.

We have to develop better antibiotics and a better methodology to achieve the final target *i.e.*, to have a man bacteria-free and fungi-free and also parasite-free. To conclude, I would say that we should go on because of the promising aspects and that we should look for further improvement of this form of therapy. This can be achieved only by further studies.

Brumfitt (London)

I remain to be convinced that the infants were made germ-free; was the technology of anaerobic culture adequate to detect all organisms?

Dr. Jameson (London)

Dr. Gaya and I have some unpublished work which suggest that you can achieve a state of reduced infection by giving non-absorbable antibiotics. Leaving the fact that you may not at the present time improve the remission rate in acute leukemia, it seems to me that there is an indication for continuing these investigations because if you might, at the moment, argue that if the patients become infected you can treat their infections with systemic antibiotics, we may not be able to stay in that happy position for very much longer, because of the emergence of resistant strains. Therefore, protection and prevention

will become more important so I think we should continue to investigate.

Phillips (London)

The thing that troubles me is that anybody can recognise aerobic organisms; but anaerobes are delicate organisms and many are very difficult to grow. Just because you can grow one does not mean you can grow the lot. Finally, some of the anaerobes are extremely oxygen-sensitive organisms and I'm sure there is very little information about them in these patients.

Gaya (London)

I would like to make one comment to Prof. Brumfitt. It does not matter if we sterilize the guts of our patients or not; what is important is to suppress the potential pathogens, or the organisms which cause trouble in these patients and I think that any of the gut-sterilizing regimens which are used do this pretty satisfactorily. To amplify a little bit of what Dr. Jameson started to say, I would like to add that our two groups had their gut flora suppressed using a mixture of framycetin, colistin and nystatin together with an orificial regimen using chlorhexidine. In short, we have reduced the number of infectious episodes in the test group, we have reduced the number of septicemias in the test group, we have reduced considerably the number of deaths and the number of pyrexial episodes was also considerably reduced. We have also reduced to a fraction the usage of systemic antibiotics, and although our remission of leukemia rate in the two groups was similar – and here I would take issue with Dr. Armstrong – the quality of life for our test group (the gut sterilized group) was certainly much better than that in the control group. •

Schimpff (Baltimore)

I do not believe that the patients within the protected environment are put on that much additional stress. It is true that the antibiotics are exceedingly distasteful and that many patients vomit or have diarrhoea and don't like them. The isolation itself is certainly distasteful to all of us, but the patients again don't seem to mind that so much. The patients in or out of the isolation room have about the same degree of anxiety and depression, so that the isolation alone does not seem to be of major importance. It seems to me at the present time there is certainly no overwhelming evidence to say that every hospital in the world should have laminar air flow rooms or use oral non-absorbable antibiotics in the leukemic patients. Those techniques should not be adopted until there is additional data showing that their use is going to make a major change in the survival of patients with acute leukemia. I think however, that these therapies should continue to be investigated.

Nauta (Leiden)

One indication for granulocyte transfusion is in the patients with no granulocytes in the peripheral blood or less than $50/mm^3$ and when there is a persistant septicemia despite adequate bactericidal antibiotics being given. We say good results in that special indication and I would ask Dr. Strickmans if in his material perhaps more significant data can be obtained if he looks at those particular patients.

Dr. Stryckmans (Brussels)

This is of course still another indication. I think one has to clearly define the different indications for granulocyte transfusions and do controlled studies. I don't see any other answer.

Levine (Bethesda)

There is an outgoing randomized study going on at the National Cancer Institute now in patients who are given or not given granulocytes for documented granulocytopenia and Gram-negative septicemia. In either group the patients are given early therapy with carbenicillin, gentamicin and cephalothin. The trial is already quite large and it shows a survival rate with antibiotics alone of 80 % and with antibiotics and granulocytes of 80 %. This supports the studies presented by Dr. Stryckmans.

Armstrong (New York)

I also suggest considering the importance of opsonising antibodies in severely infected granulocytopenic patients. We found that in Pseudomonas infections the presence of opsonising antibodies was critically important for survival.

Buckner (Basel)

When you look at randomized studies, especially like the National Institute of Health study, you are talking about very marginal doses of granulocytes; so it does not surprise me that it shows no difference.

In fact this has been shown before; if you take 5 billion cells and you give them to someone you have no difference between the groups, but that is a small number of granulocytes and you wouldn't expect it to be effective. I think that the technique is still not available for achieving the granulocyte turnover rate that we would need so I would be a little hesitant to say that the granulocytes do work or that they don't work. Actually with the cell dose we have right now, I think we should do prophylactic studies. We should

take a group of patients at high risk and give granulocytes before they become infected. If you know the infection rate is going to be high and if it does not work under these circumstances, obviously it's not going to work for someone who has Gram-negative sepsis.

Dietrich (Ulm)

I think that is a very good idea. It is a more experimental point of view and it takes into account all the notions which have been developed here.

Wrigley (London)

We have that study now started at St. Bartholomew in London on prophylactic granulocytes study in acute myeloid leukemia; we hope perhaps within 18 months time to be able to give some indication on that problem.

Medenica (Geneva)

How is your procedure for elution and did you see a difference of activity between granulocytes obtained from the filtration procedure and those obtained from the machine? Lastly, how many times can the donor be used for filtration?

Stryckmans (Brussels)

I shall first answer the question about the activity of the cells. I have no personal results to compare the activity of filtration PMN to the activity of centrifugation PMN. Results in the literature suggest that the difference is not great.

I think that nothing is really well known about what happens to granulocytes which have been obtained from donors who received corticosteroids. We do not know whether the effect of corticosteroids on the PMN seen in vivo is only the consequence of a direct cellular effect on PMN or whether a part is played indirectly by an effect on other cellular elements for instance. The "steroid lesion" of the PMN should certainly be better investigated.

We are not using the filtration leukapheresis in our Institute.

How many times a donor is used is variable from center to center. We usually do not use a donor more than 2–3 times for centrifugation. In other centers the donors are used 5 times or even more within a short period of time.

Brumfitt (London)

I would just like to ask for clarification about hyperalimentation; do you use only amino acids in the solution? How often do bacteria enter through the infusion site to cause septicaemia?

Schimpff (Baltimore)

Hyperalimentation does include amino acids. We gave hyperalimentation to 22 patients for about a 13–14 months' period with the hope that we would build them up and be able to give them chemotherapy. Among these patients, 13 of the 22 developed 18 septicemic episodes, caused mostly by *Candida albicans* and group D Streptococcus. However, hyperalimentation was not the only condition present which predisposed to these infections.

How do the organisms get there? The bottle was always sterile. I imagine, just to guess, it was something happening in connection with the catheter or perhaps the organisms came from other sites, the gastrointestinal tract, for example. Perhaps there was a predisposing factor such as the hyperglycemia or hypophosphatemia. Dr. Tattersall has mentioned that these conditions may predispose to infection in addition to the granulocytopenia each patient had.

Your second question is about the lung biopsies; I really was not referring to any type of lung biopsy. We have been using the brushing technique and we have been successful in many patients provided they have adequate platelet levels. Dr. Armstrong mentioned the open lung biopsies, others use the drill biopsy and still others use the bronchoscopic biopsy. I finally have the feeling that it probably depends on the technician; it is probably a mistake to have many individuals doing a variety of procedures instead of one person who knows how to do one procedure and can do it well with minimal complications.

Jameson (London)

Another question to Dr. Schimpff: I quite agree with you about the general use of cultures, particularly in the debilitated patients who are prone to be colonized with coliforms in the upper respiratory tract. I would like to know whether you frequently see some more irrefutable evidence of E. coli as a pulmonary pathogen. I must admit that I tend to regard it as not likely to be a pulmonary pathogen but maybe I am doing the wrong thing.

Schimpff (Baltimore)

I can remember a few cases of peneumonias with a bacteremia, due to E. coli and so I shall make the assumption that the pulmonary pathogen was the same. Beside this I think that you're right; I think that Pseudomonas and Klebsiella are very much more common than E. coli as causes of penumonia, among the Gram-negative organisms.

Phillips (London)

I just wanted to ask clarification of one point: what proportion of these patients you investigated in the proper way actually do eventually turn out to have infection?

Schimpff (Baltimore)

You're coming back to the group of patients with acute leukemia and granulocytopenia who developed a sudden fever. I used to say about 80 % and that could be broken down into about 60 % total in which there would be a well-defined infection, as microbiologically defined; the other 20 % would be less documented. I think most of us would consider it adequate to define infection clinically. The biggest problem would be to prove what the agent is in pneumonia. On the other hand, with the present E.O.R.T.C. protocol, the percent of infection which cannot be documented is in the range of 30–40%. If I can speculate for the Group, there are various possibilities to explain this, but it may well be that we are giving therapy sooner and because of earlier therapy we are not able to document some of the febrile episodes.

Buckner (Basel)

I would like to ask Dr. Schimpff if he has seen a bacteremia following his rectal examinations in patients who complain of pain in the rectal region and who have no granulocytes. In our experience, if they complain of pain they almost certainly have infection and if one does a rectal examination, especially if one does endoscopy, one is likely to end up with bacteremia. We treat these patients before we examine them on the basis of their history.

Schimpff (Baltimore)

I think there is no question that the person who is examined can develop bacteremia; we know that after sigmoidoscopy and after chewing, bacteremia may occur. The point I want to make is that if you can't see anything and if you don't know the site of infection, the excuse that the bacteremia may occur should not prevent one from doing a rectal examination in a person who has exquisite rectal pain.

Buckner (Basel)

Yes, but if you have internal pain, do you go ahead and do the examination without coverage of antibiotics?

Schimpff (Baltimore)

I think it's fair to say that the examination usually is done first, the culture is done second and then the antibiotics are started. But it's not unreasonable to start with antibiotics and then do the examination. The critical thing in my mind is not to avoid the examination and say it shouldn't be done. I think it's important to know where the infection is, so that the patients can be treated properly, including appropriate local therapy.

Dr. Pennington (Boston)

I question Dr. Zinner about the idea of using a vaccine for preventing Gram-negative infections in patients with malignancy, and of course the next question is whether immunosuppressed patients give an adequate immune response to such a lipo-polysaccharide type of vaccine? If they can respond would this be effective in preventing infection? There is a lipo-polysaccharide vaccine made from extracts of *Pseudomonas aeruginosa* which is prepared by Parke, Davis and Co. Dr. Armstrong's group has studied it with a large number of cancer patients and found a statistically significant decreased mortality from Pseudomonas infections. A group of pediatricians was also studying the vaccine in leukemia and they did not show a statistically decreased incidence of Pseudomonas infection in the vaccine group. We did a study at the National Institutes of Health, basically in adults with leukemia but with a few pediatric leukemics. We vaccinated 22 patients and found a very nice antibody response. In our study we only vaccinated patients that were in remission and not receiving chemotherapy. In this regard we got a better antibody response than in the other two studies in which patients were sometimes in relapse. Of note was that the increase in antibodies was not related to the amount of previous chemotherapy, except for methotrexate.

Also, we looked at secretory antibodies in sputum and did not find a good rise despite a good serum rise. So, there has been some work in this regard; it is a bit in dispute whether it is effective or not but I think that we should look at this Pseudomonas vaccine in more patients.

Armstrong (New York)

I would like to add a comment to Dr. Schimpff and Dr. Zinner: a skin lesion should be not only cultured but consideration should be given to a biopsy. The latter may be life-saving. I think it is terribly important to make a specific diagnosis when a skin lesion appears.

Buckner (Basel)

It wasn't clear from the discussion Dr. Armstrong, whether you thought varicella and zoster was transmissible, person to person. On the slide you said that it was, but in your discussion you disagreed with Dr. Schimpff when he said it could be a contagious disease.

Armstrong (New York)

There are a limited number of cases where a first contact with varicella resulted in clinical zoster. They are rare and I don't think we have adequate serological or virological studies to get an idea how often it occurs.

Buckner (Basel)

We have seen in our transplant patients two clusters where we had an index case and 5 to 7 contacts who developed zoster within a 1 to 3 week period; but that still does not conclude for the evidence that this is contagious; we nevertheless have to go on with some assumptions.

Schimpff (Baltimore)

In these patients, who are highly predisposed, it would appear on an epidemiological basis, as it was just mentioned, that after exposure and an incubation period somewhere around 3, maybe 4, weeks Herpes zoster may occur. We had a group of 91 individuals who were exposed to a boy who had chickenpox. Among those 91 patients, about 25 of them were at high risk; in those 25% developed Herpes zoster. This is fairly strong epidemiological evidence but certainly not proof that this was a second infection caused by the exposure as opposed to reactivation of latent virus.

Levine (Bethesda)

I'd like to ask Dr. Armstrong what he does about isolated candidemia in leukemics as opposed to the hospital population at large.

Armstrong (New York)

If they appear to be clinically well, I would usually not treat them but rather culture them repeatedly. If they appear to be clinically ill, as is usually the case, my inclination is to treat. I think it is a risk to say that Candida is just a contaminant in the blood culture or that the fungemia is only transient. As I said when I started to talk about Candida, it is one of the most difficult problems in infectious diseases to decide whether the Candida infection is invasive or not.

Jameson (London)

Dr. Schimpff, since you've started gentamicin–vancomycin–nystatin regimen for intestinal decontamination have you had a reduction of perianal sepsis?

Schimpff (Baltimore)

In that group of 34 patients receiving oral non-absorbable antibiotics, we have had only one perianal lesion compared to our usual rate which runs much higher, around 30 %. In the patients getting the oral antibiotics, there has been a definite reduction in perianal lesions. If the patients don't get a remission they stop taking antibiotics and they become recolonized and develop a lesion, but while they take the antibiotics we have seen very few perianal infections.

Phillips (London)

On this subject of anal lesions again, it's the one area where bacteriology might be very confusing: at least in our patients there is a fecal flora which contaminates the area. How do you decide which is the etiological organism?

Schimpff (Baltimore)

First of all, many of these patients develop a bacteremia; 60 % do so. In our experience, perianal infection is usually caused by Pseudomonas, occasionally by another Gram-negative organisms. Only once did we see Bacteroides and once *Staphylococcus aureus.* On rare occasions, there are true abcesses, and you can get some specific material out of this. Now, in the type of therapy we have been using since the E.O.R.T.C. program has been started there is carbenicillin and gentamicin apart and there are some combinations of those. That tends to cover most of the microorganisms that were there and the carbenicillin is adequate for most Bacteroides in addition. So there is a reasonable coverage for most of the bacteria even though only Pseudomonas is usually isolated from the blood.

Levine (Bethesda)

At the risk of pointing out the obvious I think it should be said that the perianal lesions are very much the function of the kind of chemotherapy that the patient has been on. If you put patients on methyl-GAG almost everybody gets a perianal lesion, but if you put patients on other drugs the incidence may be much lower.

Armstrong (New York)

I think that it is terribly important to separate the patients in two groups; those who go into remission when they have their infection and those who don't go into remission. Those who go into remission usually get better and those who don't usually don't. I think, in trying to figure any kind of statistics to evaluate the success of antibiotics, that the occurrence or not of remission is one very important fact one has to take into account.

Tattersall (London)

I would like to ask Dr. Schimpff what his approach is to the patients who have been colonized by hospital-acquired organisms and in whom you have shown that the risk of subsequent septicemia is so high. Do you treat them with prophylactic systemic antibiotics or what would you recommend in this situation?

Schimpff (Baltimore)

In actual fact, despite the fact that we know that certain organisms, if they colonize the patients, are very likely to cause infection, we hav'nt done any-utilized that data directly because the E.O.R.T.C. protocol defines therapy.

Gaya (London)

I'd like to take this opportunity to thank the Institut Jules Bordet and its staff for putting on this meeting and in particular I should like to thank Dr. Klastersky for all the work he has done in organizing it. I thank Beecham, England and Belgium, and in particular Dr. Pintens for supporting this meeting and for the hospitality shown to all of us.

Klastersky (Brussels)

To conclude this meeting and the corresponding discussion, I would like to present a few personal impressions. Firstly, it is clear that infection caused by Gram-negative microorganisms is a major cause of morbidity and mortality in patients with disseminated cancer.

In these patients, many factors predispose to severe infection; it is to be stressed that many conditions other than cytostatic therapy may be involved here. It seems to be the case of parenteral hyperalimentation and platelets transfusion. It means that the care of patients who undergo chemotherapy is not only the problem of chemotherapists and oncologists, but that general medicine clearly has its place in the management of patients with cancer. In other words, the practice of internal medicine should remain integrated to the general approach of the patient with cancer.

On the basis of experimental data it appeared, several years ago, that the use of protected environment and of granulocyte transfusions might be beneficial to the neutropenic individual, especially when acute leukemia was the underlying condition. Protected environment and granulocyte transfusions have both been tested under clinical conditions and now, we are left with the impression that no major advantages accrue from the use of these techniques. The reasons for these findings are unclear; and probably many causes are involved. However, one has to realise that we are not dealing, under clinical conditions, with experimental models but rather with sick people in whom variations are great from many points of view. Difficulties in the scientific assessment of new therapeutic approaches arise from the relative rarity of diseases such as acute leukemia making difficult for a single centre to accumulate a broad experience within a short period of time during which no major medical changes would have been introduced.

The need for cooperative trials is thus obvious, in spite of the variations which are necessarily introduced when multiple hospitals work together in building up larger series of patients. Maybe we have with the use of protected environment and granulocyte transfusion the possibility of helping some patients, but we still do not know who are those who will benefit most. Further studies should be devoted to delineate clearly the indications for these supportive measures.

The discussion on protected environment and granulocyte transfusion has also indicated that with systemic antibiotics we are able to cope with a substantial proportion of severe bacterial infections encountered in neutropenic patients with neoplastic diseases. The nature of the optimal regimen(s) to be used has been discussed in the past during a similar symposium held here, at the Institut Jules Bordet, in 1973*. The desire for the search of an optimal therapy has resulted in the creation, under the auspices of the E.O.R.T.C. of the Antimicrobial Therapy Project Group and in the development of a cooperative evaluation of various antimicrobial regimens, the preliminary results of which are reported in the present volume. The development of effective antimicrobial therapy thus remains the keystone of the rational management of patients with cancer and infection, especially in those with neutropenia. Thus, there is no doubt about the importance of the optimal use of antibiotics when

* Optimal antimicrobial therapy in patients with cancer. *Europ. J. Cancer*, 9, 393 (1973).

one deals with sepsis in cancer patients, but there are clearly other aspects.

A rational diagnostic approach to this type of patient is of major importance; it is clear that infection in the patient with neutropenia superimposed on the course of neoplastic disease does not present itself in the same way as does infection in other patients. The need for a special training, for a special experience in infectious diseases in cancer patients, is therefore obvious.

In addition, a certain degree of sophistication in our approach to the infected patient is necessary. When vasomotor collapse or other manifestations of severe sepsis are present, antibiotics no longer can be relied upon to save the patient; thus we need to understand better the pathophysiology, the immunology and many other aspects of infections with which we are dealing. To pay attention to this might be as important as to diagnose as early as possible and to treat as effectively as possible the infected patient.

Finally, if Gram negative infection has become a major problem in severely debilitated patients since Gram positive infections can be dealt with quite effectively, it is clear that in the patient who undergoes cytostatic or/and immunosuppressive therapy a new wave of fungal, viral and protozoan infections is now coming. We have just begun our experience with these uncommon infections which will, probably, take even more importance in the future.

To conclude this session, I would like to thank very sincerely the speakers who agreed to come and share their experiences with us today. I also thank Beecham for its generous support to this meeting and all those who participated to make it feasible and, hopefully, useful.