DOCTORIAL (Ph.D.) THESES

INFECTIONS OF FEBRILE NEUTROPENIC PATIENTS IN MALIGNANT HEMATOLOGICAL DISEASES

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INTRODUCTION

Febrile neutropenia is a frequent complication following chemotherapy in oncohematological practice. In 1966 was the first time Bodey et al. directed the attention of clinical experts for the first time to the close connection between the severity of infection and the degree and duration of neutropenia among those patients who were receiving chemotherapy for acute leukaemia.

Patients with absolute neutrophil counts lower than 500/mm$^3$ with a total neutrophil count is less than 1000/mm$^3$, especially if the number of these white cells is falling rapidly, are considered to be at increased risk of infection and mortality. Fever in neutropenic patients is a frequent complication of chemotherapy for cancer. It occurs in $> 80\%$ of those with blood malignancies. According to data from international literature, more than two thirds of febrile episodes are likely to be caused by infection with fever frequently going the only sign of infection.

At least one-half of neutropenic patients who become febrile have an established or occult infection, and at least one-fifth of patients with neutrophil counts of $<100$ cells/mm$^3$ have bacteremia. Fungi are a common cause of secondary infection among neutropenic patients who have been treated with broad-spectrum antibiotics for the primary bacterial infection. Noninfectious etiologies of pyrexia include: the underlying malignancy, chemotherapy, transfusion of blood products, and occasionally antimicrobial agents, CSF or allergic reaction. Thus, in neutropenic patients, infection should be considered the cause of fever until proven otherwise.

Febrile neutropenia following chemotherapy in patients with malignant haematological diseases is classified in three categories:

1. Microbiologically documented infections, subdivided into those with and without bloodstream infection.
2. Clinically documented infection.
3. Fever of unknown origin (FUO).
Gram-positive bacteria now account for two-third of microbiologically documented infections. About 80% of all Gram-positive bacteriemias among patients with febrile neutropenia are caused by CNS, viridans streptococci. Bactremias occur in only 15-20% of the microbiologically documented infections in febrile neutropenic patients. Gram-negative agents are more frequently isolated from urinary tract, respiratory tract and gastrointestinal tract (in connection with enterocolitis or periproctal infection). Anaerobes are an infrequent cause of microbiologically documented infections in the neutropenic host. The common fungal agents in neutropenic patients include Candida spp. and Aspergillus in prolonged neutropenia. The herpes group of viruses (herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6), and community-acquired viruses (respiratory syncitial virus, influenza virus, and parainfluenza virus) have emerged as important pathogens in selected subsets of patients (hematopoietic stem cell transplant recipients). Some of these infections have a seasonal distribution.

Because the progression of infection in neutropenic patients can be rapid, and because such patients with early bacterial infections cannot be reliably distinguished from noninfected patients upon presentation, empirical antibiotic therapy should be administered promptly to all neutropenic patients at the onset of fever. Because rapid diagnostic methods (Gram stain, antigen detection) or characteristic features of infection rarely help in the diagnosis, empirical antibiotic treatment must be considered with the goal of eradicating the most frequent organisms causing fulminant infections, that may result serious in complications (S. aureus, viridans streptococci, pneumoococci, P. aeruginosa, Klebsiella species., E. coli). Although fungal infections are usually superinfections, Candida species or other fungi can cause primary infections in some cases. In the selection of the antibiotic regimen, one should consider the type, frequency of occurrence, and antibiotic susceptibility of bacterial isolates recovered from other patients at the same hospital. The use of certain antibiotics may be limited by
special circumstances, such as drug allergy or organ dysfunction (e.g. renal or hepatic).

Two general schemes of intravenous antibiotic therapy are used: monotherapy or combination therapy. The basis of monotherapy or combination therapy is an antipseudomonal β-lactam antibiotic (ceftazidime, cefepime or piperacillin/tazobactam or imipenem/cilastatin or meropenem). The clinicians can select among aminoglycosides, either amikacin or netilmicin, and among glycopeptides either vancomycin or teicoplanin. Limited studies show that a single daily dose of an aminoglycoside with ceftriaxone is as effective as monotherapy with ceftazidime. Quinolone-based combination with β-lactams or glycopeptides are an option for initial therapy in patients not receiving quinolone prophylaxis. Newer agents (levofloxacin, moxifloxacin) have been used selectively to treat patients who have cancer. Quinupristin-dalfopristin is also effective against vancomycin-resistant Enterococcus faecium. Additional studies are needed to place the newer antibiotics in proper perspective.

Hematopoietic growth factors (G-CSF, GM-CSF) can consistently decrease the duration and degree of the neutropenia, which in turn favourably alters the course of the infection.

AIMS

2. To survey the frequency of bacteremia and the distribution of causal organisms of bloodstream infections in febrile neutropenic patients following chemotherapy in two study periods and examine susceptibilities to antibiotics which we use in the therapeutic protocol.
3. To survey the frequency of pneumonia and its role in the mortality in the two study periods.
4. To estimate the effectiveness of the antimicrobial treatment in febrile neutropenic patients following chemotherapy.
5. To establish the origin of death associated with infections in the two study periods.
6. To survey the effectiveness of antibiotic prophylaxis we applied in neutropenic patients following chemotherapy.
8. To obtain experience in combination treatment of chronic disseminated candidiasis with fluconazol and GM-CSF.

**SUBJECTS AND METHODS**

At the Department of Medicine of the Central Military Hospital of Hungarian Defense Forces I performed a study in the periods from January 01, 1995 to December 31, 1997 and from January 01 1998 to December 31, 2001. in febrile neutropenic patients with malignant haematological diseases, following chemotherapy.

**SUBJECTS**

The subject selected for the study were patients with malignant haematological disease who developed a fever (twice or more within 24 hours, at least 38°C axillary temperature, within 21 days following chemotherapy and with an absolute neutrophil granulocytes count of 500/mm³ or lower during the febrile period.

A total of 52 patient (23 women and 29 men, average 54,8 years) met the criteria – including 25 patients (23 women and 29 men, average 54,8 years) in the first period and 27 (14 women and 13 men, average age 62,2 years) in the second.
METHODS

The sample techniques

History was obtained and physical examination was performed. Complete blood count, blood chemical and coagulation tests, urinalysis and cultures of blood and urine taken before empirical antimicrobial therapy was begun. A chest X-ray was performed within 12 h of initiating therapy. At least one specimen of blood for aerobic and anaerobic cultures was drawn through the catheter lumen (if present) and another set from a peripheral vein.

Microbiological methods

The microbiological examinations of blood, sputum, urine and intravascular catheters were performed by conventional microbiological methods. biochemical identifications of the isolates were carried out by conventional methods and confirmed by the ATB system (bioMeriux). Among organisms we followed with attention to the identification of S. aureus, coagulase-negative Staphylococcus, P. aeruginosa, A. baumannii, E. coli. The antibiotic susceptibilities were first determined by disc diffusion method. The minimal inhibitory concentration (MICs) were determined by E-test (AB Biodisk) according to the manufacturer’s instructions. MICs and minimal bactericid concentrations (MBCs) were determined by the microdilution method using the “National Committee for Clinical Laboratory Standards” (NCCLS) guidelines using inoculum concentrations of approximately 10⁵ and 10⁷ colony-forming units (CFU/ml).

ESBL production was confirmed by both the double-disk synergy test and E-test.
The identification of fungi from blood cultures was performed by use of routine bottles. Serological examinations were performed cases of determination of atypical organisms (M. pneumoniae, Legionella spp.)
**The animal experiment**

*Sources and identification of strains*

The SHV-5 ESBL-producing K. pneumoniae strain was originally isolated from a premature infant intensive care unit. Bacterial strains were cultured from both inpatients and outpatients of the clinics of the Semmelweis University, Budapest and from the Perinatal Intensive Care Unit of the “Géza Hetényi” County Hospital.

*Drugs*

Ciprofloxacin (Bayer) and levofloxacin (Aventis Pharma) were freshly diluted with physiological saline before each experiment in accordance with the manufacturers’ instructions.

*In vitro susceptibility testing*

The MIC was determined by the microdilution method using NCCLS guidelines. The MBC was determined by subculturing 0.1 ml from each clear tube onto agar plates, and were defined as the lowest concentration of a drug that reduced the number of viable organisms by 99.9%. MIC and MBC were determined using inoculum sizes of approximately $10^5$ and $10^7$ colony-forming units (CFU/ml).

*Killing curves*

Initial bacterial concentration was $7.69 \log_{10}$ CFU/ml. The chosen concentrations of antibiotics were close to the in vivo mean serum levels and were 2 µg/ml for ciprofloxacin and 3 µg/ml for levofloxacin. The killing curve was calculated at 3, 6, 12, and 24 hours following incubation with antibiotics. Serial dilutions were carried out in order to eliminate the carryover effect for appropriate counting of colonies. An amount of 0.1 ml was subcultured on agar plates and incubated at $37^\circ$ C for 24 hours for the CFU determination. The lowest accurate count was $6 \times 10^3$ CFU/ml for the killing curve, i.e. 30 colonies on an agar plate on to which 0.1 ml of the 20-fold diluted sample had been subcultured.
**Animal model**

Male CD-1 mice, weighting 30 to 35 grams, were used for the pharmacokinetic study, determination of the blood bacterial counts and to perform the survival analysis.

**Pharmacokinetic analysis**

Thirty mice were chosen and used in each group, and 20 mg/kg dose of ciprofloxacin or 50 mg/kg of levofloxacin were administered intraperitoneally. Blood samples of five-five mice were taken by cardiac puncture 15 and 30 minutes and 1, 2, 3 and 4 hours after the drug administration. Antibiotic levels of sera were determined by high pressure liquid chromatography.

Values for pharmacokinetic variables were calculated by a non-compartmental method. Peak plasma concentration \( (C_{\text{max}}) \) was obtained directly from the experimental data. The terminal-phase elimination rate constant \( (K_{\text{e1}}) \) was estimated by linear regression of the \( \log_{10} \) values of concentrations. Half-life \( (T_{1/2}) \) values were calculated as \( 0.693/\text{mean } K_{\text{e1}} \). The area under the concentration-time curve from time zero to last sampling time \( (\text{AUC}_{0-4h}) \) was calculated by the trapezoidal rule, the AUC from 4 hours to infinity \( (\text{AUC}_{4h-8}) \) was estimated as \( C_{4h}/K_{\text{e1}} \), where \( C_{4h} \) is the concentration measured at four hours of treatment. The total area under the concentration-time curve from zero to infinity \( (\text{AUC}_{0h-8}) \) was determined as the serum of \( \text{AUC}_{0-4h} \) and \( \text{AUC}_{4h-8} \). The under the concentration-time curve from the zero to 24 hours \( (\text{AUC}_{0-24}) \) was calculated as 4-fold of \( \text{AUC}_{0h-8} \).

**Blood bacterial counts**

Fifteen infected mice were used in each group, with one infected untreated control group. The treatment started two hours after the infection and lasted for a total of 24 hours. Antibiotics were given intraperitoneally. The following antibiotics were evaluated: ciprofloxacin 20 mg/kg every 6 hours and levofloxacin 50 mg/kg in every 6 hours. Blood samples were taken from the tail vein at 8, 14 and 26 hours after the infection, when the antibiotic serum levels were far below the MIC values. After serial dilutions the samples were cultured quantitatively on
Mueller-Hinton agar plates. After overnight incubation at 37°C, viable bacteria were counted and expressed as the mean \( \log_{10} \text{CFU/ml} \) in blood. In case of no bacterial growth the limit of detection, i.e. 300 CFU/ml were replaced.

**Survival analysis**

Fifteen infected mice were used in each group, with the same treatment regimen as for the determination of blood bacterial counts but using two control groups: the uninfected, and the infected untreated. The follow-up period was one week looking for the survival of mice, taking deaths as the endpoint. The treatment period was assessed by the estimation of the cumulative probability using Kaplan-Meier survival curve. \( P < 0.05 \) was accepted as statistically significant.

**RESULTS AND STATEMENTS**

The proportion of the microbiologically documented infections during the two study periods among our patients with febrile neutropenic episodes was 51% (67 episodes). There were 47 cases of bacteremia (35.6%). The frequency of localised infection was 9.1% (12 cases). Our data exceed the data published in international literature, which can be explained by the higher occurrence of central venous catheter-associated infections and pneumonia, the accurate usage of antimicrobial protocols, concerning the febrile neutropenia, in addition the good cooperation with clinical microbiological laboratory. We observed clinically documented infections in 28 cases (21.2%), which correlates with the international data. In connection with microbiologically and clinically documented infections we observed central venous catheter-associated infection in 7 cases (7.4%), which is slightly higher than the rate of observed in international studies (5.3%). The rate of fever of unknown origin (FUO) was 28% (37 cases), which is significantly lower than the reported values of 43, 41 and 38%.
We observed 47 bacteremias and 2 fungemias among the 67 microbiologically documented cases. Among bloodstream infections we verified in 33 cases (70.2%) Gram-positive organisms, while we detected Gram-negative organisms in 14 cases (29.8%). In 66.6% of Coagulase-negative staphylococci we found oxacillin resistance, while all strains of CNS were susceptible to glycopeptides. In the first study period (1995-1997) the rate of Gram-positive bacteremia exceeded Gram-negative bacteremia (56% versus 40%, the remaining 4% was fungemia), but the difference was non-significant. Compared to international data in our patient population the CNS predominate. Among these organisms all *S. epidermidis* strains were methicillin/oxacillin resistant, and 3 isolates out of 7 were susceptible to vancomycin and teicoplanin. The non-*S. epdermidis* strains were susceptible to methicillin/oxacillin. These data underline the importance of empirical administration of vancomycin or teicoplanin. We started empirical antibiotic treatment with vancomycin in combination with another broad spectrum antibiotic with antipseudomonas activity in 22.5% (16 occasions) we used the glycopeptide antibiotics in cases of catheter-associated infection, cellulitis, and severe mucositis.

In the second study period (1998-2001) we observed an increasing rate of bacteremia due to Gram-positive organisms. Among the 61 febrile neutropenic episodes we detected 23 bloodstream infections (37.7%). Eighteen of these bacteremias were due to Gram-positive pathogens (78.2%). This means a 22% increase compared to the earlier study period, while the proportion of central line infections (5 versus 2 cases) and mucositis (4 versus 3 cases) decreased. This trend is very similar to the XI. trial of EORTC, when the percentage of Gram-positive bacteremia was 69%. Gram-positive pathogens, predominantly CNS and Viridans group streptococci, may now account for as many as two thirds of bacteremic episodes in febrile neutropenic cancer patients.
We observed 1 lethal outcome (*E. faecalis* sepsis) out of 18 cases of Gram-positive bacteremia (1 polymicrobial case), while 13 bacteremic cases due to CNS proved to be benign. According to international practice, in the absence of clinical signs of central venous catheter-associated infections, cellulitis or severe mucositis, we refrained from empiric usage of glycopeptides, although the length of hospital stay still increases. We must take into consideration, the extensive use of glycopeptides increase to the risk of emerging vancomycin resistant bacteria.

Among Gram-negative organisms in our study *P. aeruginosa* dominates. In the two study periods we observed 5 bloodstream infections due to *P. aeruginosa* out of 14 bacteremias, with 2 lethal outcomes. In our study, among antipseudomonas β-lactams carbapenem (imipenem/cilastatin and meropenem), there was one strain of carbapenem resistant *P. aeruginosa*, while all isolates were susceptible to ceftazidime. In our hospital in 2001 the proportion of carbapenem resistant isolates of *P. aeruginosa* from blood cultures was 18.5%, while for the ceftazidime this ratio was only 12%. For this reason in 2002 we introduced an initial empiric therapy of ceftazidime ± amikacin to our antimicrobial guidelines in neutropenic patients with fever.

During the second study period we used β-lactam (ceftazidime) + aminoglycoside (amikacin) antibiotic combination only in 5 cases of our group of patients with neutropenic fever. In this group 1 patient died.

In our study among 132 febrile neutropenic episodes following chemotherapy we observed pneumonia in 26 cases (19.7% of documented infections). This ratio is slightly higher compared to the rate in international studies (13%). Our data suggest, that pneumonia is one of the leading causes of death in febrile neutropenic patients, because in the second study periods 4 of 12 patients treated for pneumonia died. Of note, 1 of 4 pneumonia cases with lethal outcome was due to pulmonary aspergillosis, and in another case *C. glabrata* could have played the role in the development of pneumonia and sepsis, in addition
to the bacterial infection (*P. aeruginosa*). It is very important to perform of the traditional radiological examinations (chest x-ray and sinus films) early, as well as use modern imaging techniques (chest CT, MRI), and if possible to perform BAL, transbronchial biopsy and microbiological examinations.

In our study we can establish, that the response rate of the initial, empirical antibiotic therapy in the febrile neutropenic patients with bacteremia, in the two study periods was 70.2%, which means that the therapy was effective in 33 cases, and in 14 cases we needed treatment modification. The effectiveness of initial, empirical antibiotic therapy was 62.1%.

In our practice we used most frequently imipenem/cilastatin empirical antibiotic therapy; this was successful in 32 cases (52% effectiveness), while in combination with vancomycin (n=16) the response rate was 87.5% (14 patients after empirical combination therapy became afebrile, in 2 cases amphotericin B treatment was necessary). We started the empirical antibiotic therapy with vancomycin in combination with another wide spectrum antibiotic in 23 occasions according to our antimicrobial protocol. We administered amphotericin B in 9 cases, despite this treatment we lost 2 patients due to invasive fungal infections.

The 8.4% mortality rate of febrile neutropenia is similar to the data in the international literature (6-8%).

The 14.7% mortality rate due to febrile neutropenia was higher in the than earlier study period (8.4%). The mortality rate due to bacteremia was 17.4% as opposed to the earlier period, when the mortality rate in this category was 4.2%.

During afebrile neutropenia we used chemoprophylaxis in 49% of cases in our first study period, while in the second study period in 23% of cases only. For chemoprophylaxis we used ciprofloxacin. In the first study period during antibiotic prophylaxis 12 bacteremic cases (34.2%) occurred, while in the second study period we observed 6 bacteremic cases (42.8%), and CNS were the most frequent organisms among these
bacteremic cases. Fifty percent of these CNS were resistant to ciprofloxacin. Antibiotic prophylaxis, especially quinolones, can reduce the occurrence of fever, but not the rate of infection. There was a change in the spectrum of pathogens (dominancy of Gram-positive organisms). We did not find quinolon-resistant *E. coli*. Most of the guidelines recommend that routine antibiotic prophylaxis for afebrile neutropenic patients be avoided, because of the problem of emerging drug-resistant bacteria and fungi due to extensive antibiotic use. Therefore, since 2002, with this group of patients we did not use antibiotic prophylaxis. We place great importance on isolation and increased infection control activity. We start administering fluconazole prophylaxis if we notice multiple colonisation. We do not routinely administer antiviral prophylaxis.

We performed the earlier described animal experiment because of the emerging role of *P. aeruginosa* among Gram-negative organisms, emergence of carbapenem resistant ESBL-producing pathogens, and frequent use of β-lactam antibiotics in the clinical practice. The aim of our study was to determine and compare the activities of ciprofloxacin and levofloxacin against ESBL-producing *K. pneumonia* strain *in vitro* and *in vivo*. In our experiment both ciprofloxacin and levofloxacin had a good *in vitro* activity against ESBL-producing *K. pneumonia*. The low MICs and MBCs and the absence of an inoculum effect which was found to be an advantage compare to β-lactams. Ciprofloxacin and levofloxacin both decreased the bacterial count *in vitro* in the killing-curve study rapidly and permanently. Emergence of resistant bacteria was not observed. Although in some other studies ciprofloxacin was found to be more active in vitro than levofloxacin against *K. pneumonia*, in our study there was no difference in the bacterial killing rate of the two agents. In our experiment ciprofloxacin and levofloxacin had the same *in vivo* efficacy. There were no significant differences in the blood bacterial counts and in the survival rates between the two treated groups. Comparing the infected-treated groups and the infected-
untreated group there were significant difference in the blood bacterial counts and the survival of mice.

The outcomes of the treatment with fluoroquinolons has been linked to the AUC$_{0-24}$ and C$_{\text{max}}$ relative to the MIC. In our study the AUC$_{0-24}$ of ciprofloxacin and levofloxacin was similar to human values. The AUC$_{0-24}$/MIC ratios were higher than reported necessary for the bacterial eradications in animal experiments and in clinical studies.

The excellent activities of ciprofloxacin and levofloxacin in vitro and in vivo seem to be promising for the treatment of serious infections due to members of the family Enterobacteriaceae producing an extended-spectrum β-lactamase.

In recent years the number of cancer patients developing invasive fungal infections has increased significantly.

In Hungary, successful combination treatment of chronic disseminated candidiasis with fluconazole and GM-CSF during the complete remission period of acute non-lymphoid leukaemia has not been employed until now. In this case the combination therapy was the last chance for the treatment of CDC, and we are very proud of the successful clinical outcome.

CONCLUSIONS

1. During the 132 febrile neutropenic episodes following chemotherapy we observed microbiologically documented infections in 67 cases (50.8%) and clinically documented infections in 28 cases (21.2%). In the remaining 37 cases (28%) the origin of fever were the progression of the underlying disease, side effects of the drugs, and non-infectious origin.

2. Among the microbiologically documented infections (67 cases) we observed 47 bacteremias and 2 fungemias. Among bloodstream infections we verified (70.2%) Gram-positive organisms in 33 cases, while Gram-negative organisms were
detected in 14 cases (29.8%). In 66.6% (16 cases from 24) of Coagulase-negative staphylococci we found oxacillin resistance, while all strains of CNS were susceptible for glycopeptides. In one case we found an imipenem/cilastatin resistant *P. aeruginosa* strain. Among those antibiotics used in the current antimicrobial protocols we have not found multidrug resistance in Gram-negative strains or ESBL producing pathogens.

3. In our study among 132 febrile neutropenic episodes following chemotherapy we observed pneumonia in 26 cases (19.7% of documented infections) and in 5 cases they played a role in the lethal outcome.

4. The effectiveness of initial, empirical antibiotic therapy was 62.1%. After the first modification this ratio was 85.6%. (The first modification was combination of β-lactam antibiotic with antipseudomonas effect + glycopeptid.)

5. In the two study periods (1995–1997 and 1998–2001) we lost 15 patients during the febrile neutropenia, the cause of death was infection in 7 patients (in 5 cases the cause of death was pneumonia). In the first study period the mortality rate due to febrile neutropenia was 8.4%, while in the second period this was 14.7%. The higher mortality rate in the second study period was explained with older age (47 year versus 62 year) and with more episodes with deep neutropenia (23 versus 30).

6. Rate of infection was not reduced with use of antibiotic prophylaxis there was only change in the spectrum of pathogens (dominancy of Gram-positive organisms).

7. Based on our experiment the excellent activities of ciprofloxacin and levofloxacin *in vitro* and *in vivo* seem to be promising for the treatment of serious infections due to members of the family *Enterobacteriaceae* producing an extended-spectrum β-lactamase.

8. In Hungary, successful combination treatment of chronic disseminated candidiasis with fluconazole and GM-CSF
during the complete remission period of acute non-lymphoid leukaemia has not been employed until now.

**The practical utilisation of the results**

1. The infections, especially pneumonias play important role in the lethal outcome of the febrile neutropenic patients after chemotherapy.
2. The older age play important role among the prognostic factors of infections, the severity of malignant aematological diseases, the degree and duration of neutropenia. These factors limit the possibilities of chemotherapy.
3. Because of the empiric and appropriate antimicrobial treatment it is very important to surveillance of the antimicrobial susceptibility of the pathogens and to identify of resistance mechanisms in the febrile neutropenic patients.
4. The results obtained in the different science and experimental medicine suggested to use in the clinical practice.

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**List of publications**

**Related to this thesis**
Papers


International and Hungarian congresses


3. **Rókusz L.**: Invazív gombás fertőzések. MH Központi Honvédkórház, Centenáriumi Ünnepi Tudományos Ülés. 1999.06.15. Budapest