Infectious Complications in Hematology Patients:
A clinical focus on prevention

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Infectious Complications in Hematology Patients:
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infectieuze complicaties bij hematologiepatiënten

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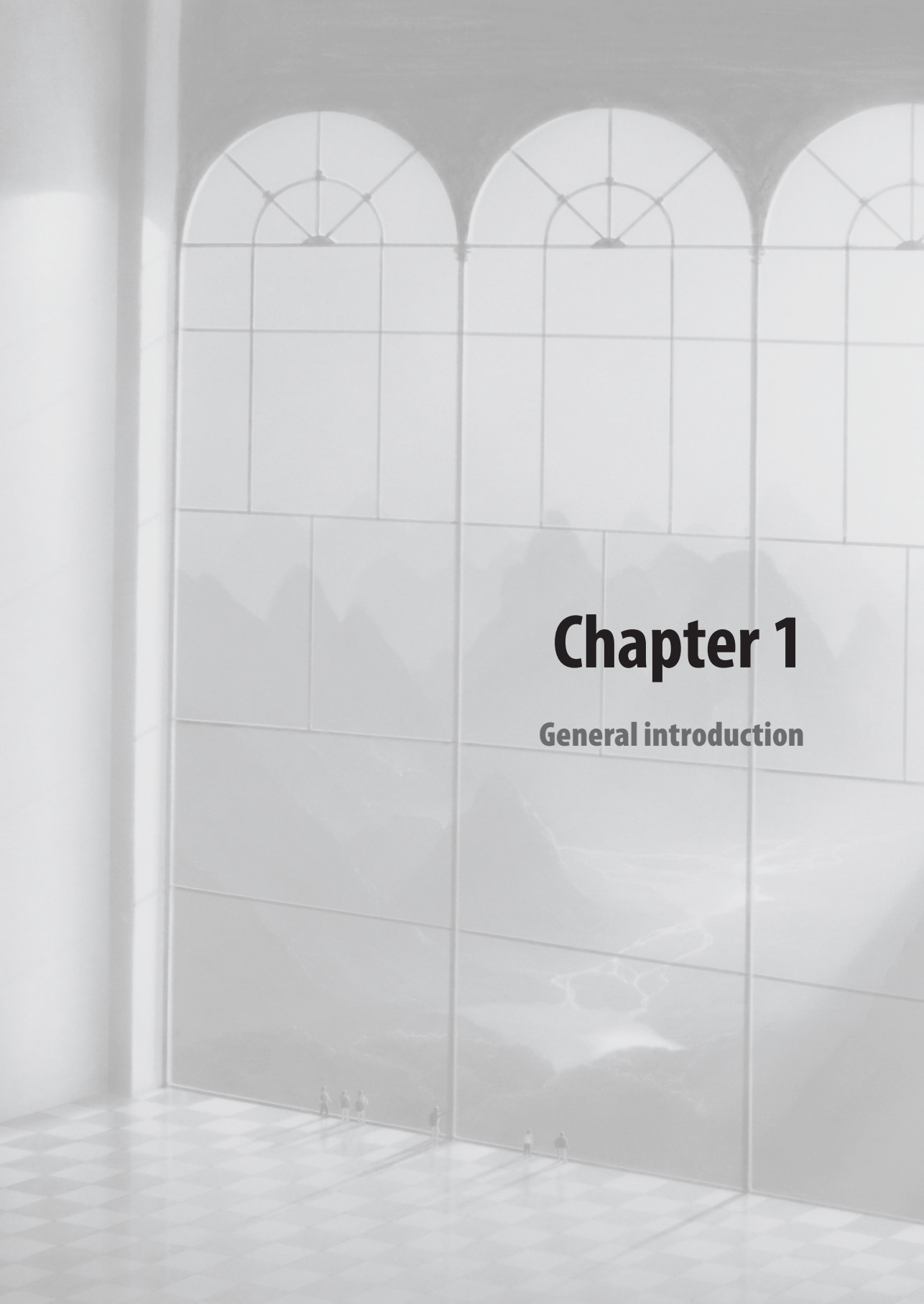

Chapter 1

General introduction

Treatment of acute leukemia: a brief historical overview

Until the 1950s and early 1960s, the diagnosis of acute leukemia was associated with a great amount of justifiable pessimism. Remissions, although in general not lasting for more than several months, were obtained with some frequency in children with acute lymphoblastic leukemia (ALL), but seldom in adults with acute myeloid leukemia (AML).[1] However, due to increasing treatment options, sequentially or in combination, the long-term survival of acute leukemia improved. Strategies including cyclic therapy, with the aim to prevent drug resistance, were developed, and the notion of multimodal therapy was considered.[2]

The close relationship between the field of hematology and infectious diseases can be demonstrated by briefly discussing some major developments of the last decennia. Interestingly, the development of antibiotics to conquer previously incurable infectious diseases has sometimes been the model for the development of anti-cancer drugs.[2] As an example, the use of anti-metabolites for the treatment of leukemia and other malignancies has been derived from the notion that sulphonamides display chemical similarity to para-aminobenzoic acid (PABA). PABA is an intermediate in bacterial folate synthesis, which is an essential nutrient for some bacteria. By mimicking PABA, sulphonamides block the enzyme dihydropteroate synthetase, which interferes with conversion of PABA to folate.

Also, some chemotherapeutic agents are directly derived from microbes. Daunorubicin, one of the cornerstones in the treatment of AML, has been derived from *Streptomyces* (*peucetius* species), the largest genus of *Actinobacteria*.

Other strategies for the treatment of acute leukemia were developed. The introduction of allogeneic bone marrow transplantation in the 1970s, using donor stem cells, was an important next step. In the conditioning phase, patients are exposed to high doses of chemotherapy, intended to reduce the tumor burden more extensively. After transplantation, the donor stem cells re-establish the blood cell production process in the bone marrow. T-cells, derived from donor stem cells, elicit an immune response that helps to destroy any remaining leukemic cells, which is called the graft-versus-leukemia effect.[3] A reduction in the early transplant-related morbidity and mortality was successively achieved by the introduction of conditioning regimens with reduced intensity (RIC), which facilitated possibilities for transplantation in older patients or patients with co-morbidity.

Progress was also made at other levels.[4] Starting in the early 1990s, the technique to collect stem cells from peripheral blood evolved. It had been observed previously that the administration of hematopoietic growth factors resulted in a huge increase

in the release of bone marrow progenitor cells into the circulation.[5] After leaving the initial reluctance to administer these growth factors to healthy donors, the technique to harvest peripheral stem cells instead of bone marrow cells has currently become the most common graft source. Under carefully selected circumstances, the possibility to use umbilical cord blood as an alternative source of hematopoietic progenitor cells has emerged since the late 1990s.[6] However, beside the worldwide increase in the number of volunteer donors, and advances in HLA-typing to identify the most suitable donor for each individual patient, one of the cornerstones enabling an increase in the number of allogeneic transplantations has been the development of effective immunosuppressive regimens for the prevention of graft-versus-host disease.

As a result of both the more effective chemotherapeutic regimens and the option of an allogeneic stem cell transplantation, the survival of patients with acute leukemia has improved significantly, especially in younger patients. For adult patients with AML, a detailed list of prognostic features has currently been developed with incorporation of cytogenetic characteristics and molecular changes. Several studies have shown that subsets of AML patients based on the expression or absence of these markers have different prognostic characteristics.[7-9] As an example, patients with translocation t(8;21)(q22;q22) or inv(16)(p13;q22) have a probability of long-term survival of greater than 60%. However, this category represents only a minority of patients. At the other hand, there is a subgroup with a less favorable prognosis, which includes nearly 25% of AML patients between 15 and 60 years old. These patients exhibit unfavorable cytogenetic abnormalities, involving ≥3 different clonal abnormalities, monosomy of chromosomes 5 or 7, deletions of the long arms of these chromosomes (del 5q or del 7q), abnormalities of the long arms of chromosomes 3 or 11, or other translocations like t(9;22) or t(6;9). AML in these patients tend to relapse in ~80%, and patients have a 5-year survival rate of less than 25%.[7,8]

More recently, various other molecular markers were identified, allowing further dissection of prognostic categories, e.g., FLT-3 internal tandem duplications (activating mutations of a hematopoietic tyrosine kinase, associated with a greater relapse risk), or nucleophosmin-1 mutations, which comprises a more favorable subset.[10-14] In the last decade, the technique of gene expression profiling, in which levels of many different mRNA transcripts are measured, has been introduced to make a further sophisticated distinct of various AML subgroups.[15] All these modifications have resulted in a refinement of the prognostic classification, from a 5-year overall survival of ~5% for patients with the worst prognosis up to >60% for patients with a favorable risk profile.[16,17]

For ALL, survival has also improved. For children, the estimated 5-year overall survival rate is around 80%. This is less favorable (35% to 40%) for adult patients.[18] Current consensus in the treatment of ALL is to use different protocols for individual well-defined risk groups, instead of one single treatment scheme for all patients.[19-23]

Treatment-related morbidity: infectious complications

The intensive treatment strategies developed to induce remission of acute leukemia were life-saving for many patients. However, these increasingly aggressive treatments resulted in a disturbed cellular defence as well as a disruption of the host's mechanical defence barriers against infectious organisms. Therefore, a rising new challenge was to deal with the infectious complications in patients treated for acute leukemia.

Intensive anti-leukemic chemotherapy, which often includes treatment with high-dose cytarabine, leads to severe and prolonged neutropenia.[24] In addition, several other patient and treatment-related factors play a role as risk factors for infectious complications, like neutrophil dysfunction, concomitant monocytopenia or lymphopenia, mucosal damage, impairment in cytokine production or interactions, and genetic polymorphisms of Toll-like receptor 4 or chemokine-ligand 10.[25-27] Furthermore, long term engraftment of allogeneic stem cells is only possible if T-cell immunodeficiency is induced with immunosuppressive drugs to prevent rejection of the transplanted graft. Suppression of T-cell function is also required to prevent graft-versus-host disease.

Over the last 2 decades, the involved microbial pathogens have changed remarkably during the incorporation of the new hematological treatment options, as the various modes of immunodeficiency put patients at changing risks. In general, the presence of (prolonged) neutropenia and chemotherapy-induced disturbance of host mucosal barriers, or other elements, like the presence of an indwelling central venous catheter may be addressed as the main risk factors for the development of bacterial infections. For bacterial infections, the incidence and severity largely depend on the duration and level of chemotherapy-induced neutropenia and mucosal damage.[28] At the other hand, the suppression of T-cell mediated immunity by various immunosuppressive agents puts patients at risk for infections with mainly viral, protozoan and fungal opportunistic pathogens.[24,25] Therefore, the risk of the development of severe viral and opportunistic infectious complications in these patients is often directly related to the level and duration of immune suppression. For the clearance of many pathogens, including yeasts and fungi, a combination of both innate immunity and T-cell mediated adaptive immunity is required.[29,30]

Thus, by the time the new hematological treatment options became incorporated, it was clear that further improvements in survival would only be possible if the diagnosis, treatment, and prevention of infectious complications of the anti-leukemic therapy were an integral part of patient management. Attention had to shift from purely treatment-related morbidity and mortality, e.g., chemotherapy-associated toxicity or acute and

chronic graft-versus-host disease, to infectious complications associated with neutropenia and T-cell dysfunction.[31]

In current hematology, infectious complications represent a dominant cause of attributable morbidity and mortality among patients undergoing high dose myeloablative chemotherapy. In a report, describing the 100-day treatment-related mortality of 1000 consecutive patients receiving high-dose myeloablative chemotherapy and peripheral blood progenitor cell transplantation, the mortality rate from infection (1.5%) was approximately half of overall mortality (3.4%).[32] Mortality in this study was low compared to other reports, but an accurate comparison with other published data is difficult, given the wide variety of criteria used to select eligible transplant candidates and the differences in stem cell sources and doses of used immunosuppressive agents.[33-38] Another study reported a mortality rate of 3.8% due to infectious complications in 314 patients treated for hematological malignancies with high-dose chemotherapy and autologous stem cell transplantation.[39] The overall rate of infectious complications until recovery from neutropenia as reported in this study was 92.3%. Microbiologically documented, mainly bacterial, infection accounted for 38.9% of febrile episodes, clinically documented infection and fever of unknown origin for 9.3% and 51.7%, respectively, of the cases. The highest probability of infection was observed for ALL and AML patients. Infection rates up to 90% correspond well with other data, although lower rates have also been published.[24,39-42]

Another recent study reported on the infectious complications in AML patients who were treated with daunorubicin and cytarabine, with (n=152) or without (n=157) the addition of cladribine.[43] Infectious complications were equally distributed in both groups, and approached 90% in this retrospective study as well. Most commonly reported were infections of the oral cavity or upper respiratory tract (~45% of all reported infectious complications in both groups). More than one third of the patients of both groups were classified as fever of unknown origin. Overall, 74 episodes (pooled incidence 26%) of bacteremia were reported, dominated by gram-positive cocci (47%, mainly coagulase-negative staphylococci), which corresponds well with other literature data.[44] In 214 of 443 febrile episodes (48%), a causative agent was documented. Of these, 132 episodes (61%) were due to bacterial infections, mainly caused by gram-positive microorganisms. Forty-three episodes (20%) were caused by proven fungal infections, mainly consisting of *Candida* species, and 7 episodes caused by *Aspergillus* species. During the induction treatment period, a total of 31 patients (11%) died due to infectious complications.

Invasive pulmonary aspergillosis

The frequency of opportunistic invasive fungal infections has significantly increased among patients with hematological malignancies or recipients of hematopoietic transplants over the last 2 decades.[45-48] The most well known infections with yeasts or fungi include *Candida albicans*, *Aspergillus fumigatus* and, increasingly, infections with Zygomycetes and hyaline molds, like *Fusarium* species. Infections range from catheter-related fungemia to more localized infections of predominantly the lungs, para-nasal sinuses, brain and skin, to widespread disseminated infection.

Aspergillus is a ubiquitous fungus that grows and sporulates in humid environments, especially on decaying organic matter. Infection with *Aspergillus* can cause a wide spectrum of diseases, although most often (80-90%) pulmonary infection, for which the inhalation of infectious fungal conidia is the first step of infection. Early reported cases of invasive infection with *Aspergillus fumigatus* date from the 1950s, concurrent with the introduction of cytotoxic chemotherapy and corticosteroids.[47] In a recent review of the therapeutic outcome of more than 1200 patients with invasive aspergillosis from 1972 through 1995, case-fatality rates of 99%, 86% and 66%, respectively, were reported for cerebral, pulmonary and sinus localisations.[49]

The host's innate immunity is of critical importance in the defence against filamentous fungi. This rapidly employed line of early defence includes non-cellular microbicidal mechanisms, like activation of proteolytic enzymes secreted along the mucosa of the respiratory tract and complement pathways. The principal cellular reaction involves uptake and elimination of infectious conidia by alveolar macrophages and neutrophils, which recognize viable conidia by means of Toll-like receptors, triggering a complex signalling cascade after contact with these foreign fungal spores.[29,30,50,51] Persistent and prolonged neutropenia, as well as treatment with high-dose systemic corticosteroids are well-established risk factors for the development of invasive pulmonary aspergillosis.[52,53] However, a definite clearing of infection requires a delayed adaptive and specific cellular immune response via T-helper cell mediated pathways, as the response to antifungal therapy alone is often inadequate.[30,54]

Despite recent diagnostic and new therapeutic strategies for invasive fungal infections and the use of high-efficiency particulate air (HEPA) filtration of the air inside the nursing rooms of high-risk patients, invasive pulmonary aspergillosis continues to be one of the leading causes of infection-related morbidity and mortality in patients with acute leukemia. Among these patients, incidence rates between 4% to 24% were reported in a 1998-survey.[47] Although the medical armamentarium to combat fungal infections has been expanded with polyenes (which, of note, have also been derived from the

aforementioned *Streptomyces*), azoles and echinocandins, mortality rates are still in the range of 25% to 50%.[49,55-57]

This has led to the development of several preventive approaches. As an example, prophylaxis with itraconazole and amphotericin B has been tried, as both formulations are active against *Aspergillus*. However, their use is hampered by various, and sometimes serious, adverse effects.[58] Recently, promising data on the prophylactic use of orally administered posaconazole were published.[59,60] However, new preventive strategies, which avoid systemic drug exposure would be welcome.

Bacterial infections and antimicrobial prophylaxis

Progress in the preceding decades has not only been made in the treatment of hematological malignancies and opportunistic infections, but also in the treatment of more common bacterial infections. In an early study from the 1960s, the mortality rate in patients with severe underlying disease, who had bacteremia due to gram-negative microorganisms, approached 90%, and was still as high as 50% in a study dating from the 1970s.[61,62] In the next years, the treatment of bacteremia in neutropenic patients improved substantially. In 8 therapeutic trials from the European Organization for Research and Treatment of Cancer-International Antimicrobial Therapy Group, performed from 1978 until 1994, the overall mortality of neutropenic patients with documented bacteremia decreased from 21% to 7%.[31] The strategy that calls for immediate empirical treatment with broad-spectrum antibiotics in patients with neutropenic fever has undoubtedly played a pivotal role in this major improvement.

The decrease in mortality reflects the successful adaptation of empirical therapy to the changing epidemiology of bacteremia in neutropenic patients. Although the overall incidence of bacteremia during neutropenic episodes did not change remarkably, a shift from predominantly gram-negative infections to gram-positive microorganisms was observed internationally during the 1980s (figure 1), although there have been indications that this etiological pattern may change again.[63]

Infections with gram-positive microorganisms became more prevalent for several reasons. First, intensification of anti-cancer treatment was associated with major impairment of the mucosal barriers, and severe mucositis. This led to an increased frequency of bacteremia with gram-positive microorganisms originating from the gastro-intestinal tract, especially the resident gram-positive oral flora. Second, the use of antimicrobials for prophylactic purposes, formerly co-trimoxazole, and later on the fluoroquinolones, should be mentioned. The selective pressure of these antibiotics led to a further shift in the etiological pattern of bacteremia.[31] Nevertheless, this prophylactic antimicrobial

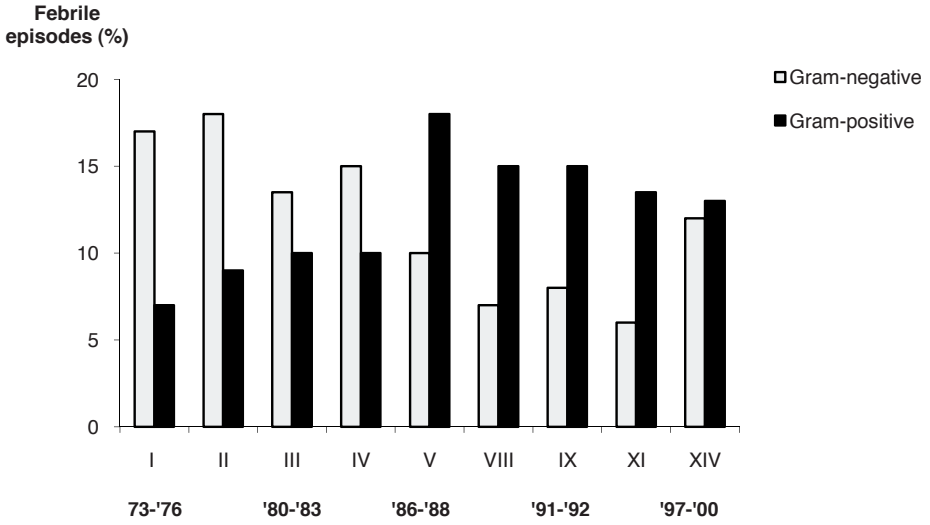

Figure 1. Bacteremia in patients with febrile neutropenia, registerd in 9 trials of the European Organization for Research and Treatment of Cancer.

treatment has proven to result in a marked reduction of bacteremia due to gram-negative microorganisms,[64-68] and even in a reduction in overall mortality, probably by lowering the intestinal burden of these microorganisms.[69] However, although gram-positive bacteria currently account for the majority of all microbiologically documented infections, the more fulminate gram-negative infections still result in a higher attributable mortality.[31,63,70,71]

Currently, antibiotic prophylaxis with fluoroquinolones, sometimes combined with non-absorbable antibiotics, during prolonged and severe neutropenia is common practice in many centers. The accompanying decrease in the incidence of gram-negative bacteremia may allow for a different therapeutic approach of patients with neutropenic fever. In case of neutropenic fever in hematology patients, current guidelines universally recommend a prolonged treatment with broad-spectrum antibiotics for 7 up to 14 days.[72] This advice is based on the assumption that most febrile episodes in these patients are due to bacterial infections. However, fever in these immunocompromised patients could just as well have a non-infectious origin. No data are available from randomized clinical trials that show that neutropenic patients, in whom evidence of a bacterial infection is lacking after a thorough initial work-up, will benefit from prolonged therapy. It may be sufficient to administer empirically started antimicrobial therapy for only a short period in patients in whom evidence for bacterial infections or bacteremia is lacking.

Catheter-related bloodstream infection

A third reason for the increase of gram-positive infections has been the increased use of central venous catheters, warranting universal bloodstream access for the delivery of intravenous medication and continuous infusion of chemotherapy and parenteral nutrition. Especially long-term tunnelled central venous catheters have become part of patient management in many modern hematology centers. However, their use puts patients at risk for mechanical, thrombotic, and infectious complications. Especially catheter-related bloodstream infection (CRBSI), mainly caused by gram-positive organisms colonizing the human skin, like coagulase-negative staphylococci, is a frequently encountered complication. CRBSI accounts for a major cause of healthcare-related bacteremia, leading to a prolonged hospital stay and significant attributable costs of patient management, even up to $35,000-$56,000 in patients at intensive care units.[53,73-77] Reported attributable mortality varies widely, ranging from 2% after correction for severity of the underlying disease until 12% to 25% in critically ill patients.[53,73,78]

Prevention of CRBSI is of utmost importance. Extensive and detailed evidence-based recommendations for CRBSI prevention were recently published.[79-81] However, many topics remain unresolved, and there is still an urgent need for efficacious preventive measures that should be easy to implement and lack the risk of developing antibiotic resistance.

And that's where we have arrived now. Thanks to a lot of pioneering work during the second half of the 20th century, treatment strategies are available for most patients with acute leukemia, although success rates vary substantially. But the improved outcome of these patients has inevitably created new hurdles. Important new challenges related to the short-term and long-term toxicity of anti-leukemic treatment have emerged. Particularly the management of serious infectious complications with considerable morbidity and mortality, and a major impact on the use of hospital resources, has become an essential part of current hematology. Therefore, the next step in the approach of these patients will be to focus, whenever possible, on infection *prevention*.

Aims and outline of this thesis

The work described in this thesis aims to contribute to the development of several preventive strategies, or to define the role of such approaches. It consists of three parts, each related to one of the major infectious complications, as discussed above.

In **Part I,** the results of several studies on the impact and prevention of invasive pulmonary aspergillosis are presented.

In **Chapter 2**, the results of a randomized, placebo-controlled trial on the prevention of invasive pulmonary aspergillosis are described. In this study, the incidence of invasive pulmonary aspergillosis in hematology patients with prolonged neutropenia receiving prophylaxis with aerosolized liposomal amphotericin B, is compared with the incidence in patients allocated to placebo.

Given the promising results of this study, it was important to consider the tolerability and various safety aspects of the prophylactic treatment with aerosolized liposomal amphotericin B. In **Chapter 3**, the results of a study on the tolerability and the effect of liposomal amphotericin B on pulmonary function are presented.

New, more effective antifungal drugs with lower toxicity profiles for the treatment of invasive aspergillosis have become available over the last years and resulted in a better prognosis. Given the limited hospital resources, up-to-date and reliable data on the diagnostic and treatment-related costs together with prognostic data of patients with invasive aspergillosis are crucial to evaluate the cost-effectiveness of any preventive strategy. In **Chapter 4**, we describe the outcome of 80 patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS), diagnosed with invasive aspergillosis during their hematological treatment. We also calculated the hospital-based management costs in these patients, and compared the results with the outcome and management costs in an aspergillosis-free cohort of 189 patients with AML or MDS.

The antibiotic strategy for the treatment of neutropenic fever developed in our hospital over the last 10 years is based on prophylaxis with fluoroquinolones and fluconazole, and differs from most international recommendations. In case of unexplained neutropenic fever, initial broad-spectrum antimicrobial therapy is discontinued irrespective of the temperature response, if no infectious origin is identified after a standardized diagnostic work-up of 72 hours. In case of a documented infectious source, directed therapy based on the susceptibility pattern of the identified pathogen is started. This has been suggested as an alternative approach previously, but the safety of such a strategy clearly needs to be evaluated in more detail.[82,83]

In **Part II, Chapter 5**, we present the results of a prospective, observational study on the causes and outcome of fever during neutropenia when this restrictive antibiotic policy is applied.

The majority of the patients treated for hematological malignancies have permanent bloodstream access warranted by an indwelling central venous catheter, for which often a long-term tunnelled catheter is used. In **Part III**, we focus on a major complication, the catheter-related bloodstream infection (CRBSI). Although diagnosing CRBSI by means

of conservative methods (i.e., with the device left *in situ*) would be highly convenient, a definitive diagnosis usually necessitates the removal of the catheter for culture of the catheter tip. However, in case of suspected CRBSI, clinicians almost invariably start antimicrobial therapy in an attempt to save the catheter, a strategy that has been demonstrated to influence the yield of catheter tip culture.[84,85]

In **Chapter 6**, we describe the results of a randomized, prospective study on the comparison of 2 techniques used to detect catheter tip colonization and related infection. In this study, both the semi-quantitative roll plate method and the quantitative sonication technique were performed on each catheter tip, which allowed us to compare the overall sensitivity of both culture methods. In the subset of patients with a clinical diagnosis of CRBSI, the diagnostic yield of both methods was determined.

In **Chapter 7**, the results of a randomized, double-blind, clinical trial on the prevention of endoluminal CRBSI are presented. We describe a strategy in which all catheter lumens of tunnelled central venous catheters were instilled with a 70%-ethanol lock solution on a daily basis during 448 catheter episodes. The effectiveness of such an approach has been described previously, but only anecdotally.

Finally, in a general discussion in **Chapter 8**, the findings of our studies are summarized and outlined against current knowledge and modern medical practice. Also, possible directions for future research are provided.

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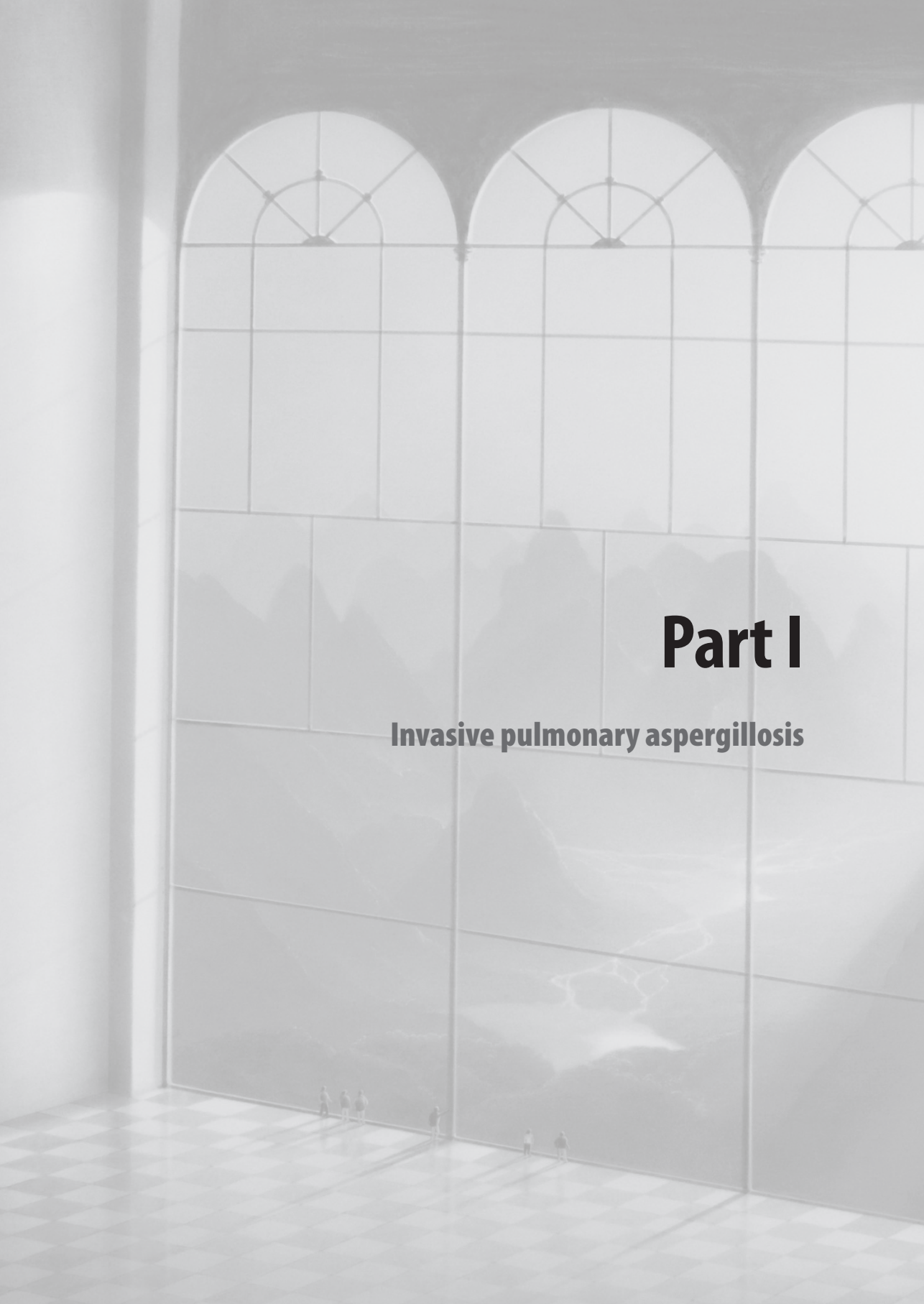
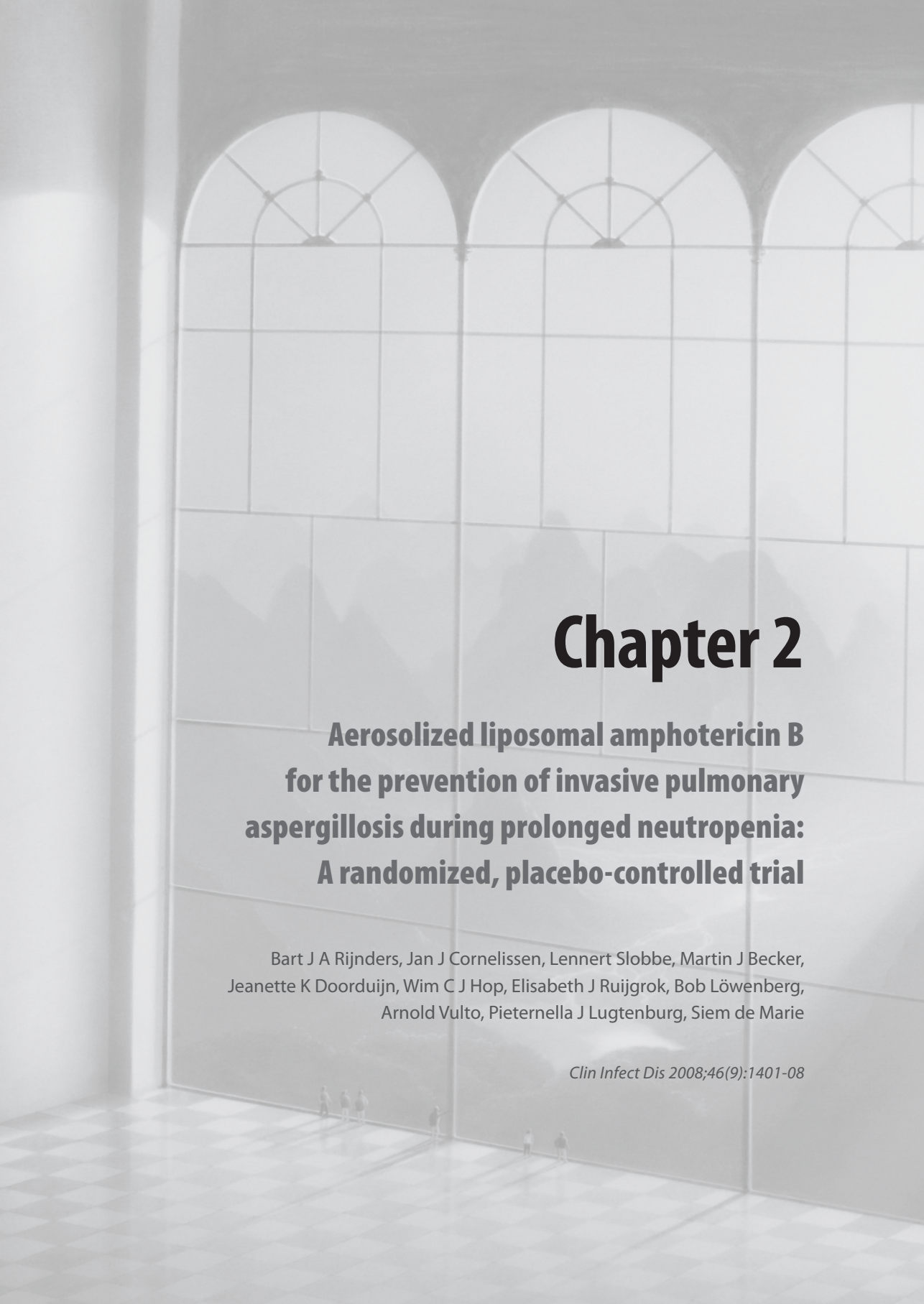


Part I

Invasive pulmonary aspergillosis



Chapter 2

Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: A randomized, placebo-controlled trial

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Abstract

Background Invasive pulmonary aspergillosis (IPA) is a significant problem in patients with chemotherapy-induced prolonged neutropenia. Because pulmonary deposition of conidia is the first step in developing IPA, we hypothesized that inhalation of liposomal amphotericin B would prevent IPA.

Methods We performed a randomized, placebo-controlled trial of patients with hematological disease with expected neutropenia for ≥10 days. Patients were randomized to receive liposomal amphotericin B or placebo inhalation twice a week, using an adaptive aerosol-delivery system, until neutrophil counts increased to >300 cells/µl. In subsequent neutropenic episodes, the assigned treatment was restarted. The primary end point was the occurrence of IPA according to European Organization for Research and the Treatment of Cancer-Mycoses Study Group definitions. Kaplan-Meier curves were compared with log-rank tests for intent-to-treat and on-treatment populations.

Results A total of 271 patients were studied during 407 neutropenic episodes. According to the intent-to-treat analysis, 18 of 132 patients in the placebo group developed IPA versus 6 of 139 in the liposomal amphotericin B group (odds ratio, 0.26; 95% confidence interval, 0.09-0.72; P=.005). According to the on-treatment analysis, 13 of 97 patients receiving placebo versus 2 of 91 receiving liposomal amphotericin B developed IPA (odds ratio, 0.14; 95% confidence interval, 0.02-0.66; P=.007). Some adverse effects, but none serious, in the liposomal amphotericin B group were reported, most frequently coughing (16 patients versus 1 patient; P=.002).

Conclusions In high-risk patients, prophylactic inhalation of liposomal amphotericin B significantly reduced the incidence of IPA.

Introduction

Invasive fungal infections are an important source of morbidity and mortality in patients receiving treatment for hematological disease. In particular, patients with prolonged neutropenia and/or severe immunosuppression are at increased risk.[1] Invasive pulmonary aspergillosis (IPA) is the most common mould infection.[2] It remains difficult to reliably diagnose IPA early; thus, several preventive approaches have been investigated. Itraconazole or amphotericin B are both active against *Aspergillus*, but adverse effects hamper their use.[3] Only recently, data on the prophylactic use of orally administered posaconazole, a new broad-spectrum azole, have become available. Compared with fluconazole, posaconazole reduced the incidence of IPA in patients with prolonged neutropenia or graft-versus-host disease.[4,5]

The inhalation of *Aspergillus* conidia is the first step in the pathogenesis of IPA. Therefore, inhalation of amphotericin B may prevent IPA while avoiding systemic adverse effects.[6] Intravenously, liposomal amphotericin B (Ambisome; Gilead Sciences) is better tolerated than conventional amphotericin B. The liposomal carrier also exhibits a pulmonary surfactant-like function.[7] In contrast, the deoxycholate salt used in conventional amphotericin B acts as a detergent, impairing the surfactant function.[8] In a recent tolerability study, more patients receiving inhaled conventional amphotericin B experienced adverse effects than those receiving amphotericin B lipid complex, another amphotericin B lipid formulation.[9] It remains unclear whether these differences in surfactant function influence the tolerability or efficacy of liposomal amphotericin B administered by inhalation.[10]

The only currently published randomized clinical trial found no protective effect against IPA for aerosolized conventional amphotericin B, but low event rates precluded definite conclusions.[11] We hypothesized that the inhalation of liposomal amphotericin B would prevent IPA.

Materials and methods

Eligible study participants were adult patients (age, >17 years) with hematological disease hospitalized at the Erasmus Medical Center (Rotterdam, The Netherlands). Patients had to start chemotherapy within 7 days after enrollment with an anticipated duration of neutropenia (neutrophil count, <500 cells/μl) of ≥10 days. This applied to patients receiving chemotherapy and patients undergoing stem cell transplantation. All patients received prophylactic fluconazole treatment. Patients were ineligible if, at entry, they already had evidence of fungal infection in their lungs or sinuses, in the case of pneumonia, if the patient was unable to use a nebulizer, if expected duration of survival was <3

months, and if the patient had previously demonstrated an intolerance to amphotericin B.

Study design

This study was a randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov identifier: NCT00263315). Randomization was performed using a computer-generated blocked list and stratified for location site, stem cell transplantation, and use of high-efficiency particulate air (HEPA) filtration. Allocation concealment was performed by employees in the Erasmus Medical Center Department of Pharmacy, who delivered syringes with liposomal amphotericin B or a placebo masked for taste, color, and optical density. Patients were randomized only once. If, after a first treatment phase with liposomal amphotericin B or placebo inhalation therapy, other episodes of neutropenia followed as part of the entire treatment plan, the patient continued the same inhalation therapy as initially assigned. An allogeneic transplantation that followed remission induction chemotherapy was considered a separate treatment entity. Therefore, inhalation therapy was not continued during the allogeneic stem cell transplantation that followed chemotherapy. Patients who had not previously entered the study could be included at the time of allogeneic transplantation. Patients discontinued participation in the study if IPA was diagnosed. The institutional review board approved the protocol, and written informed consent was obtained from all patients.

Diagnostic procedures throughout the study

Per protocol, patients underwent high-resolution CT if chest radiography findings were abnormal or at day 5 of unexplained fever despite treatment with antibiotics, and again 7 days later if fever persisted. Patients underwent bronchoscopically guided bronchoalveolar lavage (BAL) of lung lesions. The BAL specimens were cultured for bacteria, mycobacteria, and fungi, and the galactomannan level in BAL specimens was measured (Platelia *Aspergillus* EIA; Bio-Rad Laboratories). Serum galactomannan levels were measured twice weekly. In cases in which the results of these diagnostic procedures were inconclusive, biopsy specimens of lung lesions were obtained (if not contra-indicated). Intravenous amphotericin B was the treatment of choice for probable IPA until voriconazole became available (March 2002) for the treatment of IPA in The Netherlands. Per protocol, unexplained neutropenic fever for >6 days was treated intravenously with amphotericin B. Fluconazole was discontinued whenever amphotericin B or voriconazole was administered.

Inhalation therapy

Nebulization of liposomal amphotericin B and placebo was performed with an adaptive aerosol delivery system (Halolite AAD or ProDose AAD; Romedic/Medic-Aid). This is an

advanced nebulizer adapting to individual breathing patterns. It delivers aerosols only during inspiration and generates particles with an average diameter of 1.9 µm. Optimal deposition in the peripheral lung regions is therefore ensured.[12] From the start of the study until December 2003, the Halolite AAD system was used. During the second half of the study, a more user-friendly ProDose AAD system was used. Both systems generate identical aerosol particles and inhaled mass.[13]

A total of 2.5 ml of a 5-mg/ml solution of liposomal amphotericin B or placebo was used for inhalation. Nebulization was performed for 30 min per day on 2 consecutive days per week. This weekly regimen was repeated until neutrophil recovery (neutrophil count, >300 cells/µl), with a maximum of 12 inhalations per neutropenic episode.

Definitions

Two diagnostic definitions of IPA were used. For the primary efficacy end point, the European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) definitions of proven or probable invasive fungal infections were applied.[14] In brief, patients need to have a host factor, a clinical or radiological criterion (e.g., halo sign), and a mycological criterion (e.g., galactomannan detection) to be diagnosed with probable IPA. For the secondary end point of efficacy, a modified version of the EORTC-MSG definition of probable IPA was used. This definition, which has previously been applied by others, classifies patients without a mycological criterion but with the typical halo sign or air-crescent sign also as probable IPA.[11,15,16]

Outcomes

The primary goal was to compare the incidence of IPA in accordance with EORTC-MSG definitions. For the intent-to-treat (ITT) population, the follow-up period ended 28 days after neutrophil recovery from the last course of chemotherapy. For the on-treatment (OT) population, the follow-up ended 28 days after neutrophil recovery from the last neutropenic episode during which weekly inhalations were administered. The predefined secondary objective was the comparison of the incidence of IPA using the modified EORTC-MSG definitions. All end points were classified by 2 blinded investigators (B.J.R. and L.S.). Other end points were overall mortality, IPA-related mortality, and safety. Creatinine levels before the first and immediately after the last inhalation were compared to evaluate renal toxic effects. Overall mortality was registered until 28 days after neutrophil recovery. For patients with IPA, the IPA-related mortality was registered until 24 weeks after the last inhalation. Mortality was considered to be IPA-related if clinical and radiological resolution of the *Aspergillus* infection had not been documented at the time of death.

Statistical analysis

All analyses were performed on the ITT and OT populations. Patients who received at least 1 inhalation of liposomal amphotericin B were included in the ITT analysis. These patients were observed until 28 days after recovery of neutropenia after the last cycle of chemotherapy, irrespective of whether the patient was able to continue the inhalation therapy after the first course of chemotherapy. For the exceptional patient without neutrophil recovery, the follow-up continued until 28 days after the last inhalation therapy. In the OT analysis, neutropenic episodes were only evaluated in patients who received weekly inhalations during these neutropenic episodes (i.e., further observation was censored at the first neutropenic episode at which weekly inhalations were not applied).

Kaplan-Meier curves for the occurrence of IPA were constructed with neutropenic episodes as the time variable. Comparison of these curves was performed with the log-rank test, taking into account the discrete nature of follow-up evaluations (neutropenic episodes). In view of this discrete time axis, results are expressed as odds-ratios (ORs) with 95% confidence intervals, instead of conventionally used hazard ratios, which apply to a continuous time-axis. Exact calculations were performed with the LogXact program, version 4.1 (Cytel Software). Median serum creatinine levels were compared with the Wilcoxon signed rank test and proportions with Fisher's exact test. A 2-sided P value <.05 was considered to be statistically significant. We calculated that the sample size needed to show a reduction of IPA from an assumed 7% with placebo to 1% with liposomal amphotericin B inhalation with 80% power (α=.05) was 340 patients.

Results

From November 2000 through February 2006, 271 patients were enrolled and studied during 407 neutropenic episodes. During 339 episodes, inhalation therapy was administered. A total of 139 patients received liposomal amphotericin B (177 episodes) and 132 patients received placebo (162 episodes). The 2 groups were balanced for clinical and hematological characteristics (table 1). Recruitment of the planned total number of patients turned out to be more difficult and slower than expected. After >5 years of recruitment, it was believed impossible to recruit patients for another estimated 18 months. The decision to stop the study was made by the study investigators at a time when all study results were completely blinded. Because no interim analyses had been performed, the validity of the trial results was not affected.

Prophylactic effect of aerosolized liposomal amphotericin B inhalation

The analysis of the primary end point for the ITT population showed that 6 (4%) of 139 patients treated with aerosolized liposomal amphotericin B had developed IPA. A total

Table 1. Baseline characteristics of the study participants.

	Liposomal amphotericin B (n=139)	Placebo (n=132)	P
Age, mean years (range)	49 (18-73)	50 (20-74)	.64
Male sex/female sex	77/62	81/51	.33
HEPA filtration[a]	108	100	.77
Hematologic disease			
AML-MDS	65	67	.54
Other	74	65	
Hematologic treatment			
Chemotherapy	100	85	.19
Autologous HSCT	25	31	.29
Allogeneic HSCT	14	16	.70
Disease status			
Untreated	73	64	.54
Other[b]	66	68	
Treatment followed by allogeneic HSCT[c]	16	14	.85

Note. Data are numbers of patients, unless otherwise indicated. AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; HEPA, high-efficiency particulate air; HSCT, hematopoietic stem cell transplantation.

[a]Use of HEPA filtration of hospital room air during first course of chemotherapy.

[b]Partial remission, complete remission, refractory disease, or relapse.

[c]Inhalation therapy was not continued during allogeneic HSCT that followed intensive chemotherapy.

of 18 (14%) of 132 patients treated with placebo developed IPA. This difference was statistically significant (P=.005), with an OR of 0.26 (95% confidence interval, 0.09-0.72) (figure 1). Three of the 6 cases of IPA in the liposomal amphotericin B group occurred during a second or third neutropenic episode, some time after the patient had elected not to continue receiving liposomal amphotericin B inhalation therapy (43, 53 and 54 days after the last inhalation with liposomal ampotericin B). A fourth patient developed IPA shortly after he entered the study, when he had received a single liposomal amphotericin B inhalation. Until December 2003, 10 cases of IPA were diagnosed in the placebo group and 4 in the liposomal amphotericin B group. From December 2003 until the end of the study, 8 cases were diagnosed in the placebo group and 2 in the liposomal amphotericin B group. Therefore, no difference in efficacy of both aerosol systems (see "Inhalation therapy" in the Materials and Methods section) was observed.

The OT analysis showed that 15 patients had developed IPA (2 of 90 patients treated with liposomal amphotericin B versus 13 of 97 treated with placebo). This difference was statistically significant (P=.007), with an OR of 0.14 (95% confidence interval, 0.02-0.66).

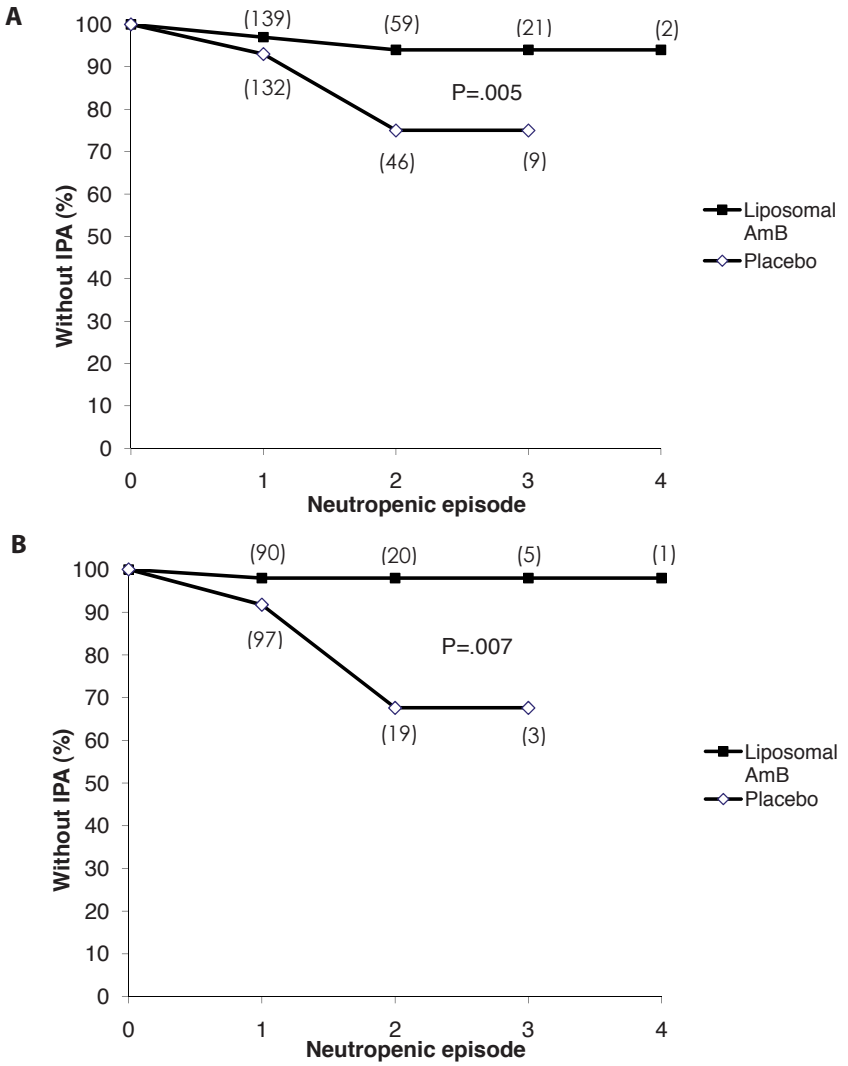

Figure 1. Kaplan-Meier curves for the occurrence of invasive pulmonary aspergillosis (IPA) according to European Organization for Research and the Treatment of Cancer-Mycoses Study Group definitions by randomized group for the intent-to-treat analysis (A) and the on-treatment analysis (B). Numbers along curves denote the number of patients studied at the various episodes. AmB, amphotericin B.

The differences between study groups for the secondary efficacy end point of IPA, using the *modified* EORTC-MSG definitions, are displayed in figure 2. For this end point as well, ITT and OT analyses showed a reduction of IPA with liposomal amphotericin B inhalation, with ORs of 0.37 (95% confidence interval, 0.16-0.83) and 0.16 (95% CI, 0.03-0.56), respectively. A total of 11 of 139 patients receiving liposomal amphotericin B developed IPA versus 23 of 132 patients receiving placebo. For the OT population,

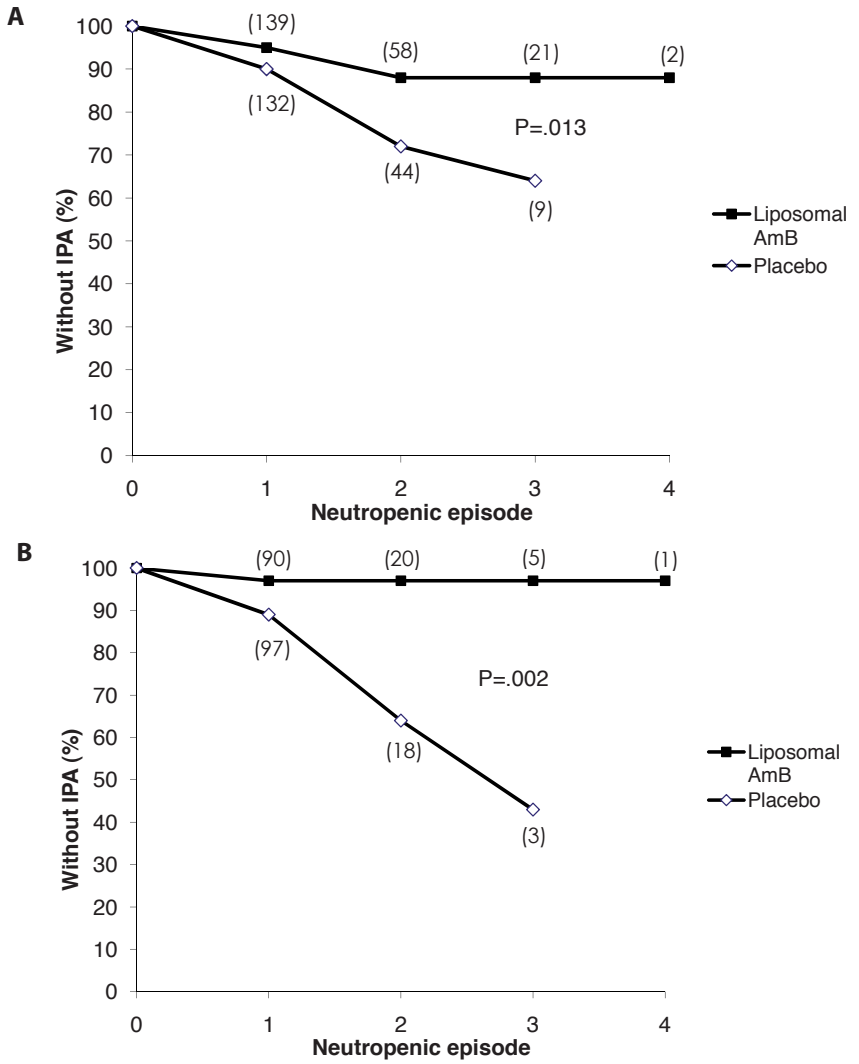

Figure 2. Kaplan-Meier curves for the occurrence of invasive pulmonary aspergillosis (IPA) according to modified European Organization for Research and the Treatment of Cancer-Mycoses Study Group definitions by randomized group for the intent-to-treat analysis (A) and the on-treatment analysis (B). Numbers along curves denote number of patients studied at the various episodes. AmB, amphotericin B.

3 of 90 patients treated with liposomal amphotericin B developed IPA versus 17 of 97 patients treated with placebo.

Only 1 case of a non-*Aspergillus* mold infection was diagnosed. This patient, who was treated with placebo, developed a fatal disseminated *Fusarium* infection. None of the allogeneic stem cell transplant recipients developed IPA.

As required by the EORTC-MSG definition of IPA, all patients with the primary end point had radiological signs of pulmonary aspergillosis. Two patients also had involvement of another site (brain, 1; cutaneous, 1). The mycological component of the primary end point definition was galactomannan detection or culture of *Aspergillus* species in the BAL specimen in 21 of 24 patients. In 4 cases, galactomannan was found in both BAL fluid and serum specimens. Serum galactomannan was the only mycological proof of IPA in 3 patients.

Survival and toxicity

Within the 28 days after neutrophil recovery, 7 patients died in the liposomal amphotericin B group (none of the deaths were IPA related), and 6 patients died in the placebo group (1 death was IPA related). The IPA-related mortality rate was 4.5% (6 of 132 patients) with placebo versus 3.6% (5 of 139 patients) with liposomal amphotericin B for the ITT population (P=.8). For the OT population, the IPA-related mortality rate was 6.2% (6 of 97 patients) with placebo versus 1.1% (1 of 90 patients) with liposomal amphotericin B (P=.1).

In the patients receiving liposomal amphotericin B, median serum creatinine levels after the last inhalation were not greater than the baseline level (59.4 and 64.9 µmol/l respectively; P=.1). In the placebo group, a trend towards increased serum creatinine levels was noted (62.7 and 74.6 µmol/l; P=.05).

During the study, significantly more patients in the liposomal amphotericin B group discontinued the inhalation therapy for at least one week (45% versus 30% in the placebo group; P=.01). The most frequently observed reasons for discontinuing treatment were the patient being too weak to use the aerosol delivery system (17 patients in both groups), a technical problem with the aerosol delivery system (12 patients with liposomal amphotericin B, 10 with placebo) and coughing during inhalation. Coughing was observed more frequently with liposomal amphotericin B than with placebo (16 patients versus 1 patient; P=.002). No drug-related serious adverse events were reported throughout the study.

Discussion

As a result of progressively more dose-intensive myelosuppressive and immunosuppressive therapy for hematologic disease, IPA represents a significant source of morbidity and mortality. In addition, IPA interferes with the delivery of the planned therapy according to schedule. Measures that effectively prevent IPA are therefore needed.

Various strategies can be considered to address this problem. Because IPA starts with the inhalation of conidia, one approach is the topical administration of an antifungal agent inside the lungs. We report the results of a study that is the first placebo-controlled

trial, to our knowledge, on the use of aerosolized amphotericin B for the prevention of IPA. Inhalation of liposomal amphotericin B reduced the incidence of IPA from 14% to 4% (P=.005). The renal toxicity of intravenous amphotericin B has hindered the prophylactic use of this drug. Inhalation therapy circumvents this problem. Avoiding systemic drug exposure also eliminates the risk of harmful drug-drug interactions. Azoles interfere with CYP P450-mediated metabolism of simultaneously administered drugs. Life-threatening interactions exist, and the observed increase in mortality with the use of itraconazole in patients undergoing allogeneic stem cell transplantation may have been the result of a cyclophosphamide-itraconazole interaction.[17] Also, systemic azole prophylaxis decreases the sensitivity of serum galactomannan monitoring for early diagnosis of IPA.[18] Amphotericin B is not absorbed systemically and therefore is unlikely to have the same effect.

In this study, galactomannan detection in BAL fluid specimens was part of the diagnostic procedure, and galactomannan was found in BAL specimens in 21 of 24 patients with IPA. In many hematology centers, galactomannan detection in BAL specimens is not a standard procedure. The modified EORTC-MSG definition of IPA also includes patients without detectable galactomannan but with a halo or an air-crescent sign on CT. Many investigators consider this radiological sign sufficiently specific for the diagnosis of IPA.[11,15,16] It is reassuring that we observed a significant reduction of IPA for both the original and the modified definitions of IPA. With the latter, a decrease in the incidence of IPA from 17% to 8% was observed among individuals assigned to receive liposomal amphotericin B treatment. Because inhaled liposomal amphotericin B may decrease the sensitivity of galactomannan monitoring in BAL, the fact that we observed a significant reduction of IPA with definitions not depending on galactomannan detection is important.

In the only published controlled study, to our knowledge, on the use of amphotericin B inhalation for the prevention of IPA, Schwartz et al. found no reduction of IPA incidence with aerosolized conventional amphotericin B treatment.[11] Several reasons may explain the lack of effect in their study. First, pulmonary pharmacokinetics and toxicity of inhaled liposomal amphotericin B used in our study may differ from conventional amphotericin B used by Schwartz and colleagues. In this regard, Griese et al. observed that deoxycholate impairs the surface tension-lowering function of pulmonary surfactant.[8] In contrast, the liposomal carrier of amphotericin B consists of phospholipids and cholesterol and exhibits a surfactant-like function.[7] Also, the inhalation system used in our study was designed to maximize efficacy of inhaled liposomal amphotericin B by targeting peripheral airways through optimal aerosol particle size and optimal lung dose. Finally, the incidence of IPA per neutropenic episode in our population was 9% (13.6% per patient) in the placebo group but only 6% in the study by Schwartz and colleagues, which limited the statistical power of the latter study. Galactomannan detection in BAL

specimens is more sensitive than galactomannan detection in serum specimens and may explain the higher incidence in our study.[15,19,20]

We observed no systemic toxicity during the study. Creatinine values in patients after the last inhalation were comparable to baseline levels. Tolerance of inhalation therapy in this ill-patient population was reasonable, but more patients treated with liposomal amphotericin B than with placebo temporarily or completely discontinued therapy. The higher incidence of cough during inhalation therapy was responsible for this difference. A total of 30% of placebo-treated patients discontinued inhalation therapy at some time during the study, which suggests that the inhalation per se was an obstacle for some patients.

Despite the higher treatment discontinuation rate with liposomal amphotericin B, the ITT analysis, which includes all patients who received at least 1 inhalation, showed a significant reduction in IPA. Additional efforts to increase compliance with the use of inhalation therapy are warranted. Ruijgrok et al. previously showed that in a rat model of IPA an improved rate of survival was observed up to 6 weeks after a single amphotericin B inhalation.[21] This finding suggests that less frequent inhalations could still be protective. Recently, the development of an amphotericin B inhalation powder was described. If tolerated well by patients, this may make inhalation therapy easier to administer.[22]

Some limitations of the study have to be noted. Allogeneic transplant recipients with graft-versus-host disease continue to be at a high risk for IPA, but these patients were not included in the study. Second, this clinical trial was halted after 62 months when 271 of the planned 340 patients had been included. Several reasons led to the decision to prematurely halt the study. The recruitment of 69 more patients would have taken a minimum of 16 extra months. Also, in 2005, promising results about the use of posaconazole for the prevention of IPA were presented. At the time the statistical analysis was performed, we discovered a higher incidence of IPA in the placebo group than was projected at the time the study was designed. This higher incidence resulted in >90% power to show the anticipated IPA reduction. Finally, this study was not designed to show a reduction in IPA-related or overall mortality with liposomal amphotericin B inhalation. Therefore, no definite conclusions can be drawn about mortality at this time, although the observed non-significant reduction in IPA-related mortality in the OT population from 6% to 1% is encouraging.

In conclusion, the results of this study suggest that inhalation therapy with liposomal amphotericin B may play a role in the prevention of IPA in patients with prolonged neutropenia. Although it was not compared directly to posaconazole and is without documented survival benefit, this therapy could present an alternative approach to IPA prevention.

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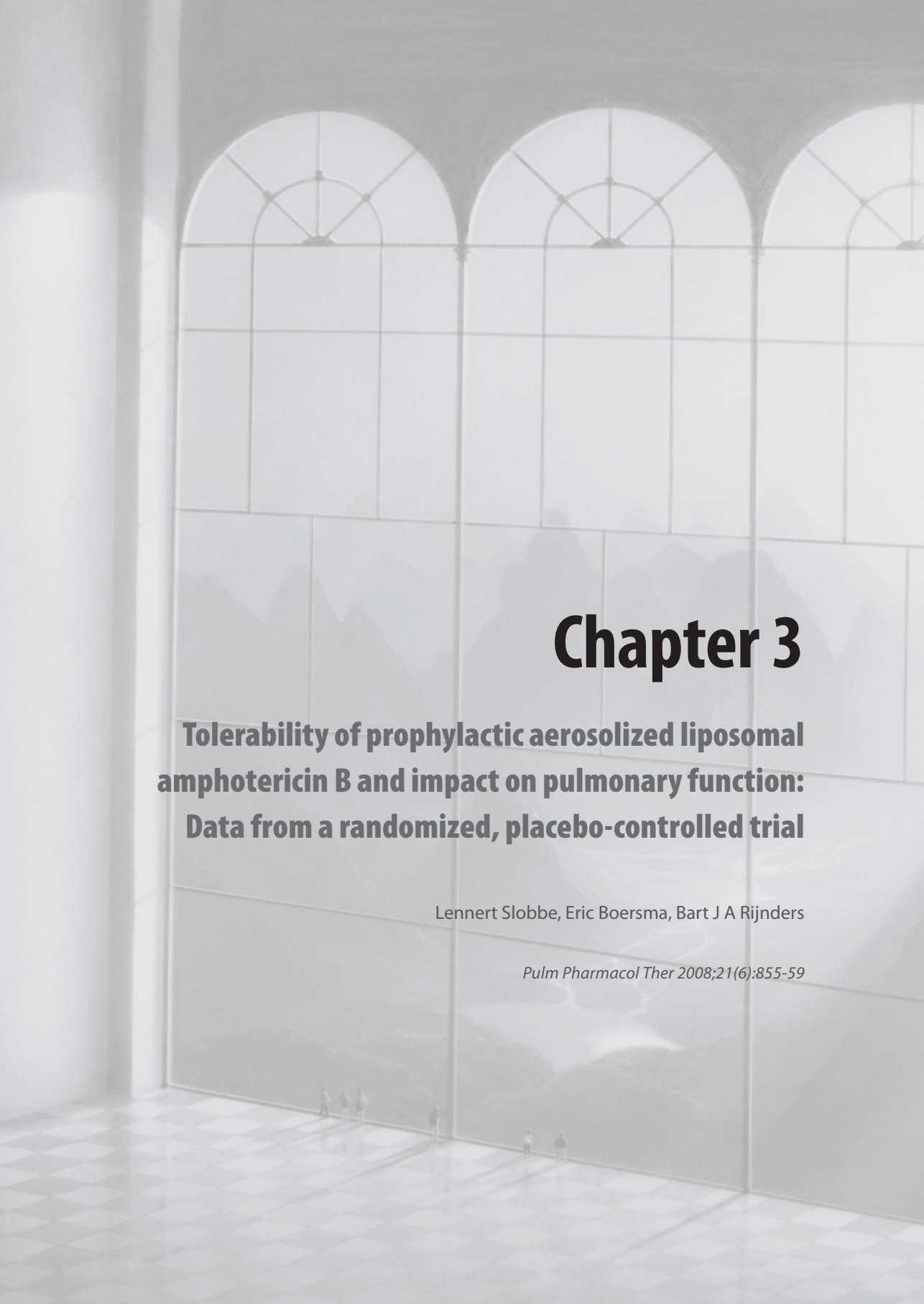

Chapter 3

Tolerability of prophylactic aerosolized liposomal amphotericin B and impact on pulmonary function: Data from a randomized, placebo-controlled trial

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Abstract

Background Invasive pulmonary aspergillosis (IPA) is a leading cause of mortality in immunocompromised patients, with the highest risk observed in patients with acute leukemia or lung transplantation. Strategies for IPA prophylaxis include administration of aerosolized amphotericin B. Liposomal amphotericin B is one of the formulations available, although few data exist on safety and tolerability.

Methods Data on tolerability, systemic toxicity, and effects of aerosolized liposomal amphotericin B on pulmonary function were recorded in a subgroup of 271 hematology patients enrolled in a placebo-controlled trial on the efficacy of aerosolized liposomal amphotericin B for the prevention of IPA. Using an adaptive aerosol-delivery system, nebulization of liposomal amphotericin B or placebo was performed during chemotherapy-induced neutropenia for 30 minutes per day on 2 consecutive days per week with a maximum of 6 weeks.

Results Thirty eight patients (41 episodes) received liposomal amphotericin B, 39 patients (49 episodes) received placebo. Proportions of patients with more than 20% post-nebulization decline in forced expiratory volume in 1 second (FEV_1) or forced vital capacity (FVC) were equal in both groups. Twenty-six of 38 patients (68%) treated with liposomal amphotericin B versus 31 of 39 patients (79%) treated with placebo had no significant decline in pulmonary function during the entire period of prophylactic nebulizations (P=.20). Coughing was significantly more reported in patients treated with liposomal amphotericin B (P<.0001). No differences were observed when baseline and post-nebulization serum levels of renal function and hepatic enzymes were compared.

Conclusions Short term prophylactic nebulization of liposomal amphotericin B was well tolerated and not associated with decline in pulmonary function or systemic adverse effects.

Introduction

The incidence of invasive pulmonary aspergillosis (IPA) among patients with hematological malignancies undergoing intensive chemotherapy or stem cell transplantation is high, varying from 5% to 24%, with mortality rates as high as 50%,[1-7] although mortality has declined during the last decade.[8-10] Several preventive approaches have been investigated.[8,11-13] Because inhalation of *Aspergillus* conidia is the first step in the pathogenesis of IPA, aerosolized amphotericin B may prevent IPA, by delivering the drug directly to the site of fungal infection while avoiding systemic adverse effects. Data from animal studies suggest that lipid formulations have advantages over conventional amphotericin B (deoxycholate), like increased pulmonary deposition and retention.[14-18] Similarly, nebulized liposomal amphotericin B showed greater prophylactic efficacy in immunocompromised mice inoculated with *Aspergillus* conidia. In a recent trial on the prophylactic use of aerosolized liposomal amphotericin B during neutropenia, we found a significant reduction of IPA from 14% to 4% (P=.005)[12,19]

Given these promising results, it is important to consider tolerability and safety in more detail. Fewer adverse events in patients on a lipid instead of a conventional formulation of amphotericin B have been reported,[20] but in a recent study, no differences were observed in tolerability for aerosolized liposomal versus conventional amphotericin B.[21] In a subset of patients enrolled in the aforementioned trial, additional pulmonary spirometry was performed on top of the registration of overall adverse events, to study these aspects in more detail. This subset was based on the random admission to 1 of our 3 hematology units, at which the lung function measurements were planned before the trial started. The present study evaluates the tolerability and effects on pulmonary function of aerosolized liposomal amphotericin B in these patients.

Materials and methods

Patient population and study design

The randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov identifier: NCT00263315) was performed at the Erasmus Medical Center, a university referral hospital with 3 hematology wards at 2 locations in Rotterdam, The Netherlands. Eligible patients were adults (age, >17 years) with hematological malignancies, receiving intensive chemotherapy or hematopoietic stem cell transplantation within 7 days after enrolment. Anticipated duration of neutropenia (neutrophil count, <500 cells/μl) had to be ≥10 days, and expected survival of >3 months, with further details described elsewhere[12]. Before the trial started, we decided to measure pulmonary function in the subset of patients who were (randomly) admitted to 1 of our 3 hematology wards. In this way, a subset of

77 of the total of 271 enrolled patients underwent lung function measurements, and data on tolerability and safety were recorded. Other information obtained included age, sex, underlying malignancy, anti-leukemic treatment response, type of stem cell transplantation, duration of neutropenia, presence or absence of COPD, and smoking habits (current smoker versus past or never smoker). The institutional review board approved the protocol, and written informed consent was obtained from all patients.

Nebulization procedures

Nebulization of liposomal amphotericin B or placebo was performed with an adaptive aerosol-delivery system (Halolite or ProDose AAD; Romedic/Medic-Aid). These advanced nebulizers adapt to individual breathing patterns, and generate particles with an average diameter of 1.9 µm only during inspiration, ensuring optimal deposition in the peripheral airways of the lungs.[22,23] From the start of the study until December 2003, the Halolite AAD system was used. During the second half of the study, a more user-friendly ProDose AAD system was used. Both nebulizer types are equivalent in terms of drug deposition and particle size.[23] Nebulization of 2.5 ml of a 5-mg/ml solution of liposomal amphotericin B or placebo was done for 30 minutes per day on 2 consecutive days per week in spontaneously breathing patients. This weekly regimen was repeated until recovery of neutropenia, with a maximum of 6 consecutive weeks per neutropenic episode. Inhalation of study medication was terminated when systemic antifungal therapy was started. If necessary, pre-nebulization inhalation of salbutamol was allowed. The color-, taste- and opalescence-masked placebo solution consisted of 5 mg of folic acid, 2 mg of quinine-sulfate, and 0.3% intralipid in glucose 5%. About 50% to 60% of this solution is assumed to be delivered to the lower respiratory tract. Quinine-sulfate has been safely used in other nebulization studies,[24] and the quantity of folic acid is expected to be non-toxic. Intralipid is present in very small amounts; its main compound consists of soja-lecithine (phosphatidylcholine), which is non-toxic, inert, and compatible with human lung surfactant components.

Data collection

Measurement of pulmonary function was performed with a portable turbiflowmeter (Micro Spirometer; Micro Medical Ltd.), which has a proven long-term accuracy and reliability.[25] During the procedure, patients were in the upright position, if possible, and encouraged by a qualified nursing team member, in order to create measurements as reproducible as possible. Pre-nebulization baseline values of forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) were recorded, using the maximum value of 3 repeated measurements. This same procedure was followed when FVC and FEV_1 were measured, both immediately after and 30 minutes after the nebulization ses-

sions in the first week. This was repeated in the fourth week, provided that patients were still on inhalation prophylaxis.

Outcome

The primary goal of this study concerning safety and tolerability aspects of aerosolized liposomal amphotericin B, was to compare the incidence of a significant decline in pulmonary function in both groups. For the primary endpoint, all measurements with a post-treatment decrease of >20% of baseline values of FVC and/or FEV_1, were identified. Taking into account the seriously ill patient population in which pulmonary function measurements were gathered under circumstances as described above, reporting absolute values would suggest greater accuracy than could be really derived from the results. Therefore, we used this relative decrease as an endpoint, which is a commonly accepted measure in other pulmonary function studies. In both groups, the presence or absence of coughing, dyspnoea, bad taste, nausea, vomiting, and dysphagia during nebulization were assessed as secondary outcome parameters. In addition, pre-nebulization baseline levels of serum creatinine, potassium and hepatic enzymes (AST, ALT, alkaline phosphatase, bilirubin and g-glutamyl transpeptidase) were collected, and per patient compared with the first value measured within 3 days after the last nebulization. For comparison, the Common Toxicity Criteria (CTC), version 2, of the National Cancer Institute were used.[26]

Statistics

Dichotomous variables were expressed as numbers and percentages. Differences in the incidence of primary and secondary endpoints between patients allocated to liposomal amphotericin B or placebo were analysed by chi-square tests or Fisher's exact tests, as appropriate. For overall estimation, we also compared the proportion of patients in both groups without any significant decline in pulmonary function during the entire inhalation period. Subsequently, logistic regression analyses were performed, to study differences in both groups after adjustment for possibly modifying smoking habits. Statistical analyses were performed using SPSS, version 13.0 (Chicago; IL). All statistical tests were two-sided, and a P value of <.05 was considered as statistically significant.

Results

Between November 2000 and January 2006, 77 patients were enrolled in this sub-study on the pulmonary safety, and followed during a total of 90 neutropenic episodes in which nebulization therapy was administered. Thirty-eight patients (41 episodes) received liposomal amphotericin B inhalation and 39 patients (49 episodes) received placebo. Characteristics of the study population are summarized in table 1. Overall, the 2

groups were well-balanced for clinical and hematological variables. No differences were observed in the length of time for which nebulization was performed in both groups.

Due to termination of prophylactic nebulization after recovery from neutropenia, the rate of patients who were still using prophylactic nebulization decreased during the study period. However, this decrease was equally divided in both groups: 17 of 39 (44%) patients receiving placebo versus 14 of 38 (37%) patients receiving liposomal amphotericin B (P=.71). Observed proportions of decline in pulmonary function after nebulization, as compared to pre-inhalation baseline values, did not differ between groups. A total of 26 patients (68%) administered to liposomal amphotericin B remained free of any significant decline in pulmonary function during the entire treatment episode, as compared to 31 patients (79%) randomized to placebo inhalation (P=.20). Per inhalation session, these percentages varied from 0% to 17%, but never with significant differences between liposomal amphotericin B and placebo inhalation. No differences were observed between both groups when taking into account the magnitude of any recorded pulmonary function decline. The results were not influenced by the smoking status of the patients. Also, the results were not influenced when corrected for the fact whether patients did already witness a significant decline in lung function in any previous nebulization session or not.

Table 1. Characteristics of the 77 hematology patients.

	Liposomal amphotericin B (n=38)	**Placebo** (n=39)
Age, mean (range)	39 (18-66)	44 (21-67)
Male sex	22 (58)	21 (54)
COPD	0 (0)	0 (0)
Hematological disease		
AML-MDS	13 (34)	14 (36)
Others	25 (66)	25 (64)
Disease status		
Complete remission	24 (63)	26 (67)
Partial or no remission	14 (37)	13 (33)
Hematological treatment		
Chemotherapy	20 (53)	20 (51)
Autologous SCT	7 (18)	5 (13)
Allogeneic SCT	12 (32)	16 (41)
Neutropenia (mean, days)	23	21

Note. Date represent numbers (%) of patients unless otherwise indicated. Two patients were treated with chemotherapy and stem cell transplantation on different neutropenic episodes; therefore, totals in hematological treatment exceed 100%. AML-MDS, acute myeloid leukemia-myelodysplastic syndrome; SCT, stem cell transplantation; neutropenia, neutrophil count, <500 cells/µl.

An overview of the tolerability data of the administered study compound is shown in table 2. There were no statistically relevant differences in the incidence of dyspnea, nausea and vomiting, neither in difficulties with swallowing between patients randomized to liposomal amphotericin B or placebo. In both groups, no pre-treatment with salbutamol was required. Coughing was significantly more frequently reported in patients receiving liposomal amphotericin B (P<.0001). In contrast, patients randomized to placebo inhalation more often complained about a repugnant taste.

Table 2. Tolerability of study compound.

	Liposomal amphotericin B (n=38)	**Placebo** (n=39)	**P**
Cough	28 (74)	10 (26)	<.0001
Dyspnea	0 (0)	2 (5)	.25
Nausea	3 (8)	6 (15)	.25
Vomiting	2 (5)	4 (10)	.35
Bad taste	14 (37)	24 (62)	.03
Dysphagia	0 (0)	2 (5)	.25

Note. Data represent numbers (%) of patients.

There were no relevant differences between liposomal amphotericin B and placebo arms when baseline versus post-nebulization serum levels of renal function and hepatic enzymes were compared. In both groups, a serum potassium between 2.5 and 3.0 mmol/l (i.e., grade 3 level of toxicity) was measured in 1 patient. Two of 38 patients on liposomal amphotericin B versus 4 of 39 patients on placebo were graded as a CTC-level 2 for creatinine values, and in the liposomal amphotericin B arm 1 patient was reported with a level 3 toxicity.[26] None of those values indicated statistical differences; this also applied for all analyses concerning hepatic enzymes.

Discussion

Our study showed that prophylactic treatment with aerosolized liposomal amphotericin B is safe and well tolerated by hematology patients with prolonged neutropenia. As IPA continues to be an important issue in these patients, all current and future developments aimed at prevention of IPA will be welcomed, with aerosolized liposomal amphotericin B as a promising candidate.[12,14,19,27] Therefore, the evaluation of tolerability and safety of liposomal amphotericin B when used as prophylaxis is needed.

Although in different patient categories, several other studies reporting on tolerability and safety of aerosolized lipid-based formulations of amphotericin B have been published.[20,21,27,28] In a randomized trial among 100 lung transplant patients, comparing conventional aerosolized amphotericin B (deoxycholate) with amphotericin B lipid complex for prevention of invasive fungal infections, Drew et al. observed treatment-limiting adverse effects in 6 of 49 patients receiving conventional amphotericin B versus 3 of 51 patients allocated to amphotericin B lipid complex (P=.31).[20] However, patients receiving conventional amphotericin B more often experienced an adverse reaction (29% versus 14%, P=.02). Treatment with 25 mg conventional amphotericin B versus 50 mg amphotericin B lipid complex (with doubled dose if mechanical ventilation was necessary) was given once daily for 4 consecutive days after lung transplantation, and was continued once a week during the next 7 weeks. In a retrospective study among 38 lung transplant patients, Lowry et al. observed adverse events during 1.0% of 1206 nebulization episodes with aerosolized conventional amphotericin B versus 1.2% of 1149 nebulizations with liposomal amphotericin B.[21] Although these numbers are encouraging, the study was retrospective with a relatively small patient population. Ruiz et al. reported good tolerance of preventive aerosolized liposomal amphotericin B in patients with hematological malignancies, but again in a population with small sample size.[27] In this study, 15 patients received 24 mg of liposomal amphotericin B 3 times per week for 1 week, followed by therapy once weekly. Premature treatment discontinuation was not necessary. In an open label study of nebulized amphotericin B lipid complex in 51 lung transplant patients, partially conducted in mechanically ventilated patients, Palmer et al. reported nausea, vomiting, and taste alteration in 3 of 381 nebulization sessions.[28] No cough and dyspnea were observed, but inhalations had to be discontinued in 1 patient due to treatment intolerance. In this study, a decline in FEV_1/FVC-ratio of at least 20% was observed in 13 nebulization sessions (4.9%) in the 10 non-intubated patients.

In general, these studies report good overall safety and tolerability of aerosol therapy with lipid formulations of amphotericin B, comparable to the results presented here in neutropenic patients with prolonged neutropenia.

The observed increased incidence of a bad taste and the non-significant higher incidence of nausea and vomiting in patients inhaling placebo solution may have been caused by the quinine-sulfate compound, which was required for blinding due to the bad taste of amphotericin B.

In terms of subjective adverse events reported in our study, only coughing was significantly more often reported in patients on liposomal amphotericin B inhalation (74% versus 26%). Reassuringly, this did not lead to severe bronchospasm, as there was no difference in decline in FEV_1 values in patients on liposomal amphotericin B compared to placebo. Also, no pre-nebulization use of salbutamol was recorded. The exact mecha-

nism of the observed increased rate of coughing is not clear. Local, but rapidly reversible, irritation of the upper airways may be an explanation. Coughing during inhalation therapy has been previously reported for other drugs, like pentamidine and tobramycin, which suggests a non-liposomal amphotericin B specific mechanism.[29,30] However, we think that the lack of severe systemic adverse events, e.g., fever, chills, renal toxicity, and/or hypotension, observed during intravenous administration of liposomal amphotericin B clearly outweighs cough as an adverse effect.

As discussed, 68% of patients administered to liposomal amphotericin B remained free of a significant decline in pulmonary function during their entire nebulization episode. This leaves 32% of patients in whom a decline was observed at some time during prophylactic nebulization. This rate of decline was 21% in patients on placebo. Taking this difference into account, 2 considerations should be kept in mind. First, a non-negligible part of patients on placebo solution also experienced a decline in FEV_1 at some time point. Second, and more important, a decline in pulmonary function during previous nebulization sessions did not predict a decline in later sessions. In case of liposomal amphotericin B as the causative agent, a re-challenge would have been expected to show the same, or even a worse, decline in pulmonary function. Therefore, it is more likely that other factors might explain a substantial part of these numbers, e.g., these patients being too weak to perform proper lung function measurements, combined with the lack of optimal circumstances.

There were no differences between liposomal amphotericin B and placebo when comparing systemic adverse events by means of baseline versus post-nebulization serum levels of renal function and hepatic enzymes. Again, considering the fact that some systemic toxicity was also reported in patients with inert placebo inhalation, this is likely to be explained by the use of simultaneously administered systemic co-medication.

Several limitations of our study should be noticed. Unfortunately, no amphotericin B serum levels were measured in patients on liposomal amphotericin B inhalation. Demonstrating undetectable serum levels after nebulization of liposomal amphotericin B, as other research groups did, would have strengthened the conclusions about the lack of systemic toxicity.[17,21,28,31] Second, performing lung function measurements in this very ill patient population under non-optimal circumstances has its limitations, as already mentioned. We tried to solve this problem by comparing post-nebulization values with baseline values measured immediately before each inhalation. Although it is conceivable that absolute values of the measured parameters may differ from measurements under optimal conditions, differences from pre-nebulization baseline values are likely to be reliable. Third, prophylactic nebulization of liposomal amphotericin B in this study population was performed while patients were in their treatment-related neutropenic

episode, with a maximum of 6 weeks. This seems to be satisfactory for the purpose of IPA prevention, as we demonstrated, but no conclusions can be drawn about long-term safety of liposomal amphotericin B on pulmonary function. Finally, the original power calculation was based on the number of patients needed to demonstrate the efficacy of prophylactic liposomal amphotericin B for the prevention of IPA, and therefore based on the primary endpoint of the efficacy part of our study. For practical and logistical reasons, the pulmonary function measurements for safety aspects were performed only in a random subset of patients.

In conclusion, the results of this study suggest that short term prophylactic nebulization of liposomal amphotericin B has no clinically significant effects on pulmonary function or systemic adverse events, and is well tolerated. Together with the efficacy, as demonstrated earlier, this supports a role for aerosolized liposomal amphotericin B in the prevention of IPA in patients with prolonged neutropenia.

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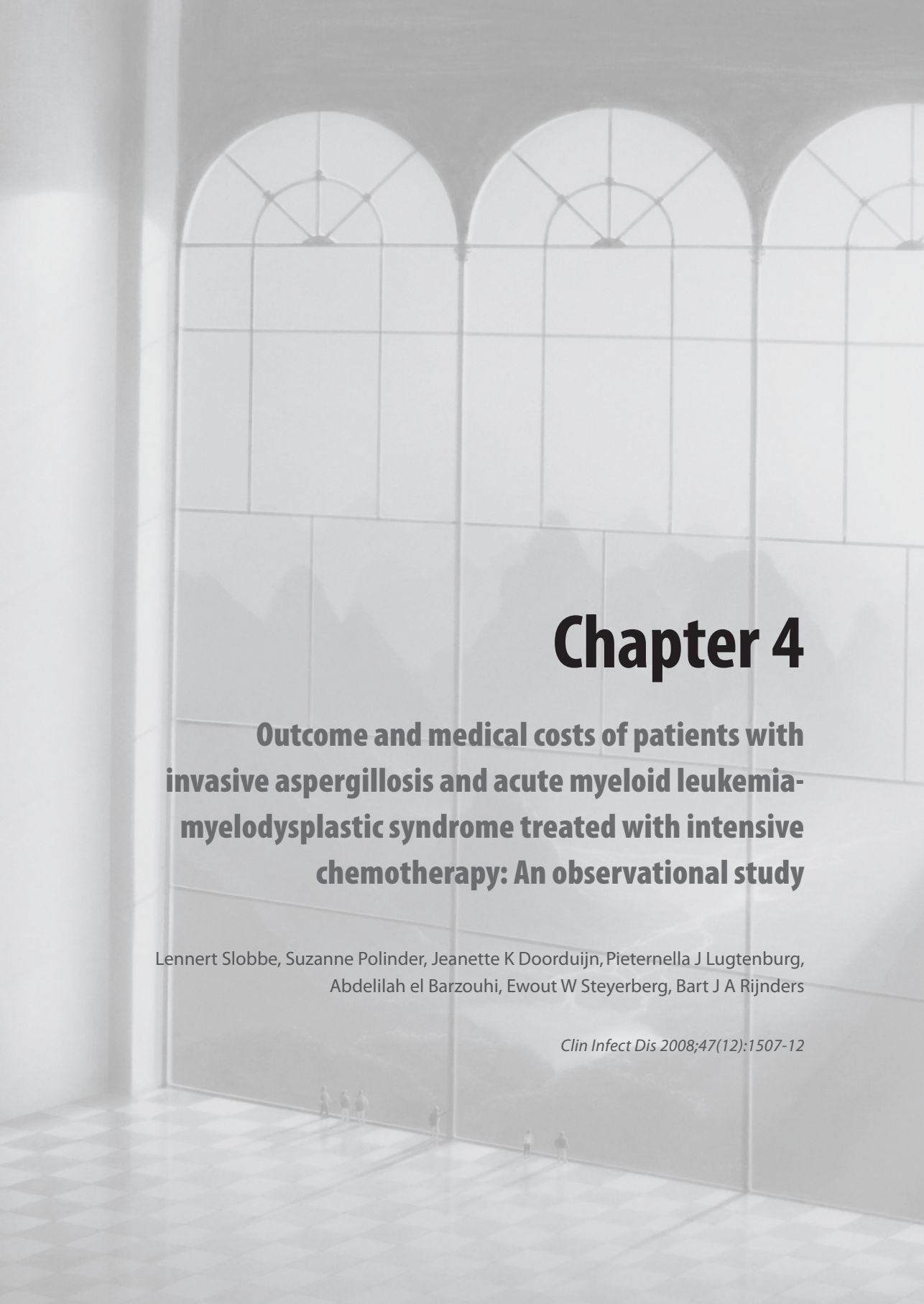

Chapter 4

Outcome and medical costs of patients with invasive aspergillosis and acute myeloid leukemia-myelodysplastic syndrome treated with intensive chemotherapy: An observational study

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Abstract

Background Invasive pulmonary aspergillosis (IPA) is a leading cause of mortality in patients with acute leukemia. Management of IPA is expensive, which makes prevention desirable. Because hospital resources are limited, prevention costs have to be compared with treatment costs and outcome.

Methods In 269 patients treated for acute myeloid leukemia-myelodysplastic syndrome (AML-MDS) during 2002 to 2007, evidence of IPA was collected using high-resolution CT and galactomannan measurement, preferably in bronchoalveolar (BAL) fluid specimens. IPA was classified on the basis of updated European Organization for Research and Treatment of Cancer-Mycoses Study group (EORTC-MSG) definitions. Outcome of infection was registered. Diagnostic and therapeutic IPA-related costs, corrected for neutropenia duration, were comprehensively analyzed from a hospital perspective. Voriconazole treatment was given orally from day 1 if possible.

Results A total of 80 patients developed IPA; 48 (18%) had probable or proven infection, and 32 (12%) had possible IPA. Seventy-three patients were treated with voriconazole; 55 (75%) took oral voriconazole from day 1. In patients with IPA, the mortality rate 12 weeks after starting antifungal therapy was 22% (16 of 73 patients). The overall mortality rate, registered 12 weeks after neutrophil recovery from the last dose of anti-leukemic treatment, was 26% in patients with IPA versus 16% in patients without IPA (P=.08), reflecting an IPA-attributable mortality rate of 10%. In a Cox-regression analysis, IPA was associated with an increased mortality risk; hazard ratio, 2.4 (95% confidence interval, 1.3-4.4). Total IPA-related costs increased with €8360 and €15,280 for patients with possible and probable or proven IPA, respectively, compared with patients without IPA (P<.001).

Conclusions Early diagnosis and treatment of IPA with oral voriconazole result in acceptable mortality rates. Nevertheless, IPA continues to have substantial attributable mortality combined with a major impact on hospital resource use, so effective prevention in high-incidence populations has the potential to save lives and costs.

Introduction

Invasive pulmonary aspergillosis (IPA) is a leading cause of infection-related morbidity and mortality in patients treated with high-dose chemotherapy for acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Published incidence rates of IPA differ among institutions and varied between 5% to 24% in a 1998-survey.[1] The reported incidence is partly influenced by the underlying disease and the diagnostic strategy followed, but environmental and nosocomial factors are likely to play an important role as well. Overall mortality rates of >50% are typically reported for IPA.[2-7] More recently, new diagnostic strategies, including high-resolution CT of the lungs and serial serum galactomannan measurement for the evaluation of neutropenic fever were introduced and had a positive impact on IPA-related mortality.[8] Furthermore, the use of chemoprophylaxis and the availability of new antifungal drugs for the treatment of IPA have been documented to decrease both the incidence and mortality of IPA.[9-11]

Given that hospital resources are not infinite, reliable data on the diagnostic and treatment-related costs of IPA and its outcome are crucial to evaluate the cost-effectiveness of any IPA-preventive strategy, apart from other aspects that need to be taken into account (e.g., toxicity and costs of any preventive intervention). The objective of the present study was to evaluate the IPA-related outcome and costs in adult patients with AML-MDS treated with intensive chemotherapy in recent years.

Materials and methods

Patient population

The study was performed at the Erasmus Medical Center, a university referral hospital with two locations in Rotterdam, The Netherlands. All consecutive adult patients (age, >17 years) with AML-MDS receiving high-dose chemotherapy during the period June 2002 through November 2007 were studied. All patients had been enrolled in a Hemato-Oncologie voor Volwassenen Nederland (HOVON) treatment protocol[12] for the treatment of AML or MDS after providing written informed consent. Information obtained included age, sex, AML-MDS risk classification,[13] number of chemotherapy courses, type of hematopoietic stem cell transplantation, duration of neutropenia, and leukemia treatment response[14] Because all patients participated in a prospective HOVON treatment protocol, the overall mortality rate was well documented and was measured 12 weeks after neutrophil recovery (neutrophil count, >500 cells/μl) from the last AML-MDS treatment-related neutropenic episode (including hematopoietic stem cell transplantation, if appropriate) and also 12 weeks after the start of antifungal therapy in patients with documented IPA.

In accordance with the diagnostic protocol for the evaluation of neutropenic fever, patients underwent high-resolution CT at day 5 of unexplained fever despite treatment with antibiotics, with repetition 5 to 7 days later if fever persisted. In the event of documented intrapulmonary abnormalities, patients underwent bronchoscopically guided bronchoalveolar lavage (BAL) of the most representative lung lesion. BAL fluid specimens were cultured for bacteria, mycobacteria, and fungi, and the galactomannan level in BAL fluid specimens was measured (Platelia *Aspergillus* EIA; Bio-Rad Laboratories). If bronchoscopy was impossible to perform because of the location or small size of the lung lesion, the galactomannan level was measured in serum specimens. If these diagnostic procedures were inconclusive, a biopsy of the lung lesions was performed, if feasible and not contraindicated. Intravenous amphotericin B was the treatment of choice for probable IPA until voriconazole became the standard of care at our hospital (end of 2002). All patients were receiving 200 mg of oral fluconazole prophylaxis per day.

Patients were categorized on the basis of recently updated European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) definitions.[15] In these criteria, a neutropenic AML-MDS patient is considered to have possible IPA if a new and otherwise unexplained well-defined intrapulmonary nodule (with or without halo sign), an air-crescent sign, or a cavity within an area of consolidation is radiologically documented. Probable IPA is diagnosed when on top of these radiological findings microbiological proof of *Aspergillus* infection is documented by antigen detection or culture of *Aspergillus*. Proven IPA was defined as histopathological evidence of invasive mold infection and microbiological proof of *Aspergillus* infection. Patients with >1 episode of IPA during hematological treatment were classified according to the highest diagnostic IPA category (e.g., a patient with possible IPA during the first course of chemotherapy but probable IPA at a later point in time was categorized as having probable IPA).

Cost calculations

Costs were studied from a hospital perspective.[16,17] Costs that were taken into account were the total hospital-based costs per patient that were likely to be influenced by the presence or absence of IPA, and/or are known to be an important contributor to overall costs of patients with AML-MDS. We distinguished diagnostic costs, costs of medical and surgical treatment, and costs of hospital stay on intensive care units and non-intensive care units. Also included were costs related to the transfusion of blood products (red cells, platelets, and/or fresh frozen plasma), because this is a frequently applied and expensive treatment in the patient category studied. Diagnostic costs taken into account were radiological imaging performed during the evaluation of unexplained fever or suspected IPA, microbiological investigations (blood cultures, staining and culture of sputum or BAL fluid specimens, galactomannan detection in serum or BAL fluid specimens),

bronchoscopy, and CT-guided or open lung biopsy. Costs of medical treatment included antifungal and antibiotic costs for the treatment of neutropenic fever. Antibiotics were also included because most patients with IPA have fever and will therefore receive more antibiotics for a longer duration, compared with patients without IPA. Surgical costs were (partial) lung resection (e.g., in patients with extensive lung tissue destruction or severe lung bleeding caused by IPA). Extramural costs included were limited to prolonged use of antifungal drugs and radiological imaging during follow-up of IPA. The evaluation was patient based and started from day 1 of the first course of anti-leukemic therapy for newly diagnosed or first relapse of AML-MDS, which means that for each patient the costs of all consecutive treatment episodes (remission inducing, consolidation, and transplantation) were taken together.

In The Netherlands, a detailed fee-for-service system is used for the remuneration of medical interventions and diagnostic procedures,[18] enabling the calculation of micro-costs. Therefore, medical costs were calculated by multiplying the volumes of healthcare use per patient with the corresponding official Dutch unit prices for each diagnostic or therapeutic procedure. For drugs, the actual number of milligrams administered was multiplied by the costs per milligram, as charged by the hospital pharmacy. The costs for inpatient days were calculated by multiplying the number of days with the unit price as charged for a ward or intensive care unit, counting the hotel costs only. We reported costs in Euros for the year 2007. Discounting was not relevant because of the limited time horizon (maximum, 6 months) of patient management.

From the point of view of making a comparison of IPA-related cost differences between patients who had AML-MDS with and without IPA, some diagnostic and therapeutic interventions were decided to be less relevant. These were not explored,[16] for example, because they were low in price or volume or unlikely to be influenced by the presence or absence of IPA. Examples are drug costs of analgesics, anti-emetics, and enteral or parenteral nutrition.

In the study performed by Herbrecht et al., voriconazole was given intravenously for the first 8 days of therapy.[9] Because of the excellent oral availability of voriconazole, patients in our hospital are treated with oral voriconazole from day 1 onward, using the same loading dose regimen used in the pivotal trial as long as severe mucositis does not preclude oral intake. Therefore, in a one-way sensitivity analysis, we estimated the additional costs when intravenous voriconazole would have been used for the first 8 days.

Statistics

Statistical analyses were performed using SPSS, version 12.0 (Chicago; IL) and STATA, version 10. Treatment costs for the IPA categories were compared using the non-parametric Kruskal-Wallis test. The number of chemotherapy courses and the AML-MDS risk classification were tested as predictors of total IPA-related costs in a linear regression analysis.

The duration of neutropenia is a well-known risk factor for IPA. Therefore, in a separate analysis, IPA-related costs were adjusted for the number of neutropenic days with linear regression analysis after exploring non-linearity of the effect of neutropenia with logarithmic, square, and square-root transformations. Overall mortality 12 weeks after recovery from neutropenia was compared between groups with the chi-square test. Differences in survival between patients with and without IPA were studied in a Cox proportional hazards model with *Aspergillus* classification as a time-dependent variable, because development of IPA occurred during the follow-up of patients. In this analysis, the status of the *Aspergillus* covariate changed over the time at the date of IPA diagnosis. Patients were censored 12 weeks after neutrophil recovery from the last treatment episode. For all analyses, a two-sided P value <.05 was considered to be statistically significant.

Results

During the period 2002 through 2007, 269 adult patients with AML-MDS received intensive chemotherapy or a stem cell transplantation during a total of 569 treatment episodes. Characteristics of the study population are summarized in table 1. A total of

Table 1. Characteristics of the cohort of 269 patients with acute myeloid leukemia-myelodysplastic syndrome treated with intensive chemotherapy, 2002-2007.

Age, mean years (range)	53.6 (18-77)
Male sex	145 (53.9)
Acute myeloid leukemia risk classification	
Low	24 (8.9)
Intermediate	115 (42.8)
High	130 (48.3)
Duration of neutropenia of >10 days[a]	267 (99.2)
Stem cell transplantation	
Allogeneic	74 (27.5)
Autologous	18(6.7)
Remission after treatment	
Complete remission	211 (78.4)
Partial or no remission	58 (21.6)
IPA classification[b]	
No IPA	189 (70.3)
Possible IPA	32 (11.9)
Probable or proven IPA	48 (17.8)

Note. Data are no. (percentage) of patients, unless otherwise indicated. IPA, invasive pulmonary aspergillosis.

[a]Neutrophil count, <500 cells/µl.

[b]IPA was defined according to updated European Organization for Research and Treatment of Cancer-Mycoses Study Group definitions.

211 patients (78%) had a complete remission of their hematological disease. A total of 267 patients (99%) had a mean neutropenic episode that lasted >10 days. Seventy-four patients (28%) underwent allogeneic stem cell transplantation. The overall incidence of IPA was 30% (80 of 269), with 32 patients (12%) having possible IPA and 48 (18%) having probable or proven IPA. IPA was diagnosed in 59 (74%) of the 80 patients before complete remission of AML-MDS was documented. Only 3 (4%) of the 80 patients developed IPA during hematopoietic stem cell transplantation, all 3 after allogeneic transplantation.

Voriconazole became the first-line treatment of IPA at the end of 2002. To evaluate overall mortality for patients treated during the voriconazole era, we excluded 6 of the 80 patients (2 with possible IPA and 4 with probable IPA) who were treated before voriconazole became available. One other patient was excluded because itraconazole instead of voriconazole was administered. In patients with IPA, the overall mortality rate registered 12 weeks after starting antifungal therapy was 22% (16 of 73 patients). Mortality in patients with possible (7 (23%) of 30 patients) or proven or probable IPA (9 (21%) of 43 patients) was comparable (P=.81). For the whole AML-MDS patient cohort, the overall mortality rate, when measured 12 weeks after neutrophil recovery from the last course of anti-leukemic treatment was 26% (19 of 73) among patients with IPA and 16% (30 of 189) among patients without IPA (P=.08). In a univariate Cox regression analysis, IPA was associated with increased mortality risk (hazard ratio, 2.4; 95% confidence interval, 1.3-4.4).

The mean additional per-patient costs were €10,530 for patients with possible IPA and €25,550 for patients with probable or proven IPA, compared with the 189 patients without IPA (P<.001). More details on the distribution of costs among different IPA categories are given in table 2. Costs for diagnostic procedures were higher for patients with probable or proven IPA (€4220) than for patients without IPA (€2240). The presence of IPA also resulted in significantly higher medication and transfusion related costs. The mean duration of hospital stay was 84 days for patients without IPA, compared with 91 and 104 days for patients with possible and probable or proven IPA, respectively (P<.001). To evaluate the robustness of the results, we re-analyzed the costs by taking together patients without IPA and those with possible IPA as a control group. The results of this analysis remained essentially unchanged, with mean costs (±SD) of €59,280 ± €23,410 in the control group and €83,300 ± €32,190 in the proven or probable IPA group (P<.001).

In a separate analysis, IPA-related costs were adjusted for the number of neutropenic days by use of linear regression, which enables the calculation of costs truly attributable to IPA. In table 3, the mean medical costs per patient are presented after correction for the duration of neutropenia. Costs of neutropenia were an additional €700 per day. After correction for neutropenia, IPA-related additional costs were €8360 for patients

Table 2. Mean medical costs per patient, according to invasive pulmonary aspergillosis (IPA) classification.

	Mean cost, €			
	No IPA[a] (n = 189)	**Possible IPA[a]** (n = 32)	**Probable or proven IPA[a]** (n = 48)	**P[b]**
Diagnostics				
Total	2240	3630	4220	<.001
Radiological	1380	2140	2450	
Microbiological	860	1490	1760	
Medication				
Total	2430	5770	7090	<.001
Antifungals	1450	4460	5780	
Antibiotics	980	1310	1310	
Hospital stay				
Total	39,450	44,120	51,760	<.001
Ward	38,020	41,100	46,500	
Intensive care unit	1430	3020	5270	
Transfusions	13,620	14,750	20,070	<.001
Other costs[c]	10	10	160	
Total costs	**57,750**	**68,280**	**83,300**	**<.001**

Note. Costs were rounded off in decades of Euros; therefore, small differences may exist when adding up totals.

[a]IPA was defined according to updated European Organization for Research and Treatment of Cancer-Mycoses Study Group definitions.

[b]Patients with no IPA versus those with probable or proven IPA.

[c]Other costs included IPA-related therapeutic and/or diagnostic costs (e.g., CT-guided biopsy, video-assisted thoracoscopy, and pulmonary wedge resection).

with possible IPA and €15,280 for patients with probable or proven IPA. The number of courses of intensive chemotherapy and the AML-MDS risk category did not contribute independently to the IPA-related costs (p>.20). Therefore, no further adjustments for these parameters were made.

Of the 73 patients treated with voriconazole, 55 (75%) were able to start treatment with oral voriconazole from day 1. A sensitivity analysis showed that systematically use of intravenous voriconazole for the first 8 treatment days would have increased the antifungal costs with another €2560 and €2680 for patients with possible or proven or probable IPA, respectively (P<.001).

Discussion

This study describes one of the largest case series of IPA among patients treated for AML or MDS, a well-established high-risk population. The overall incidence of IPA in this

Table 3. Mean medical costs per patient according to invasive pulmonary aspergillosis (IPA) classification, corrected for duration of neutropenia.

	Mean cost, €			
	No IPA[a] (n = 189)	**Possible IPA[a]** (n = 32)	**Probable or proven IPA[a]** (n = 48)	**P[b]**
Diagnostics	990	2310	2620	<.001
Medication	650	3890	4810	<.001
Hospital stay	14,930	18,140	20,370	<.01
Transfusions	4520	5110	8420	<.001
Other costs[c]	40	40	190	<.001
Total costs	**21,130**	**29,490**	**36,410**	**<.001**

Note. Costs were rounded off in decades of Euros; therefore, small differences may exist when adding up totals. Neutropenia was defined as a neutrophil count <300 cells/µl.

[a]IPA was defined according to updated European Organization for Research and Treatment of Cancer-Mycoses Study Group definitions.

[b]Patients with no IPA versus those with probable or proven IPA.

[c]Other costs included IPA-related therapeutic and/or diagnostic costs (e.g., CT-guided biopsy, video-assisted thoracoscopy, and pulmonary wedge resection).

population was 30%; 18% of those had probable or proven infection. The reported incidence is in the upper range of data published in literature,[1] which might be explained by several factors. First, patients were followed during the entire anti-leukemic treatment, including stem cell transplantation. In other studies, the duration of follow-up was shorter (e.g., until remission of the underlying leukemia had occurred).[11] Second, bronchoscopy was systematically performed in patients with intrapulmonary lesions, and galactomannan levels could therefore be measured in BAL fluid specimens. Galactomannan measurement in BAL fluid specimens has been reported to compare favorably with serum measurements for the diagnosis of IPA in this and other patient populations[19,20] and to have good diagnostic performance.[21,22] This is a likely explanation for the higher number of patients with probable IPA then with possible IPA in our case series, because the systematic use of bronchoscopy for galactomannan measurement in BAL fluid specimens will allow the upgrade from possible to probable IPA in many patients.

A significantly increased IPA-related mortality risk was seen, indicated by a hazard ratio of 2.4 in a Cox regression analysis. To compare our results with data from recent literature on the treatment of IPA, we also studied the all-cause mortality 12 weeks after the start of antifungal treatment. In patients in whom IPA had been treated with voriconazole, an overall mortality rate of 22% (16 of 73 patients) was reported. We chose to report overall and not IPA-attributable mortality, because the exact cause of death is often difficult to determine in a patient with IPA. However, 12 (75%) of the 16 patients who died

within 12 weeks after IPA was diagnosed, had uncontrolled or progressive leukemia. As a consequence, the rate of mortality that was truly attributable to IPA may be substantially lower than 22%. A difference in overall mortality of 10% was seen between patients with IPA (mortality rate, 26%) and patients without IPA (mortality rate, 16%) 12 weeks after neutrophil recovery from the last course of chemotherapy or stem cell transplantation. This comparison of overall mortality 12 weeks after the end of all anti-leukemic treatment may be a good estimate of IPA-attributable mortality.

Recently, Wingard et al. made the observation that IPA-related mortality tends to occur in the first 6 weeks after the commencement of antifungal therapy, whereas later mortality might be caused by concomitant leukemia and other factors.[23] When we re-evaluated the mortality data 6 weeks after starting antifungal therapy in our 73 patients with IPA, we found 8 case fatalities (11%) instead of the observed number of 16 (22%) at 12 weeks. If the observation made by Wingard and colleagues is true, these data are an additional confirmation that the IPA-attributable mortality rate in our study was ~10%.

We also compared our data with numbers mentioned in other studies. Several recent studies have reported all-cause 12-week mortality for IPA. In the study performed by Herbrecht et al., voriconazole was given intravenously for a minimum of 8 days and followed by oral voriconazole for as long as 11 weeks. The overall mortality rate in this more heterogeneous study population was 29% at 12 weeks and was 42% when amphotericin B deoxycholate was given.[9] In the AmBiLoad study, the 12-week mortality rate was 34%; 29% with 3 mg/kg of liposomal amphotericin B and 42% when 10 mg/kg was used.[24] The case definition of probable IPA in these 2 studies also included patients with a halo sign or air-crescent sign but without microbiological proof of IPA. This accounted for 34% of the population in the study by Herbrecht and colleagues and even 59% in the AmBiLoad study. To comply with the updated EORTC-MSG definitions, in our study the term "possible IPA" was used for comparable cases (i.e., patients with AML-MDS with radiological but no microbiological evidence of IPA).[15] Finally, caspofungin treatment resulted in a 12-week mortality rate of 46%.[25] In the study that reported on this, 85% of patients had neutropenia at study entry, strict IPA-criteria were used (all had probable or proven IPA), and 75% of patients had uncontrolled leukemia at baseline, which makes this a less favorable patient population.

In summary, the mortality rates for IPA in patients treated with oral voriconazole seem to be at least comparable to the results of the aforementioned studies.

It is reassuring that the AML-MDS risk classification and the number of treatment courses were not associated with IPA-related costs. This finding confirms that the diagnostic and therapeutic costs that we took into account did not correlate with the severity of the underlying leukemia or with the duration of hematological treatment per se, but were more specifically related to the presence or absence of IPA, as has been reported before

in a somewhat different cohort of immunocompromised pediatric patients.[26] After correcting for the duration of the neutropenia, the increase in costs in our study were €8360 and €15,280, respectively, for patients with possible and probable or proven IPA compared with patients with AML-MDS who did not have IPA, which is substantial. It remains unknown which patients should best be regarded as the control group. Including all patients without probable or proven IPA in the control group may underestimate the costs of IPA because some of the patients classified as having possible IPA really will have had IPA. However, our results seem to be robust because the observed attributable costs remained essentially unchanged regardless of the selected control group.

When intravenous voriconazole would have been used for the first 8 days of therapy in all patients, costs would increase to €10,840 and €17,890 for possible and probable or proven IPA, respectively. To our knowledge, these data are the first on diagnostic and therapeutic management costs of IPA.

We specifically focused on the costs of treating the patient with IPA from a hospital perspective. The economic consequences of IPA-related morbidity and mortality from a societal point of view (e.g., productivity losses) were not taken into account. Extramural costs were also only partially evaluated; we only focused on extramural costs of radiological imaging and use of antifungal agents.

This study has several limitations. The time frame of our study was relatively long, although AML-MDS treatment did not change substantially during this period. Because this study was observational, no definite conclusions on the efficacy of oral voriconazole versus intravenous voriconazole can be drawn. Finally, voriconazole serum level monitoring only recently became available in our hospital. Therefore, no data are available on the correlation of voriconazole serum levels with treatment response and mortality.

Considering the morbidity and mortality of IPA as well as the IPA-related costs, the most efficient strategy would be the prevention of this infection both for the individual patient and from a hospital perspective. The superiority of posaconazole for IPA prevention when compared with fluconazole or itraconazole in patients with AML-MDS has already been demonstrated.[11] Recently, we demonstrated the efficacy of aerosolized liposomal amphotericin B as a promising alternative.[27] However, a formal cost-effectiveness analysis of these prophylactic interventions remains to be established in a high IPA-incidence population, combined with early diagnosis and treatment of IPA.

In conclusion, early diagnosis of IPA and treatment with oral voriconazole therapy from day 1 resulted in a 12-week overall mortality rate of 22%. However, the attributable mortality is still substantial at 10%, as are IPA-related costs. Therefore, effective prevention in patient populations with high IPA incidence has the potential to save lives and a substantial amount of resources.

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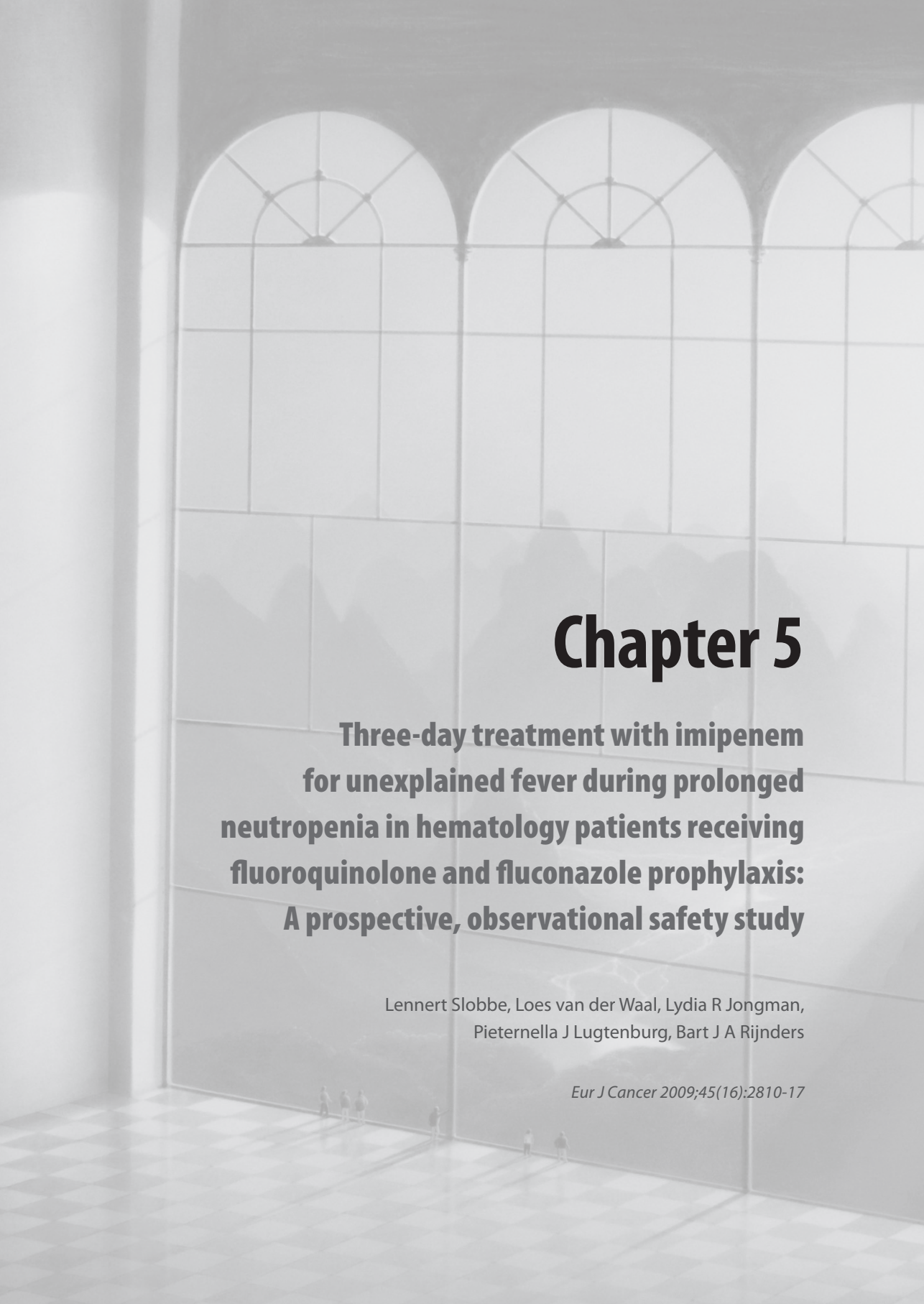
Part II

Fever during prolonged neutropenia



Chapter 5

Three-day treatment with imipenem for unexplained fever during prolonged neutropenia in hematology patients receiving fluoroquinolone and fluconazole prophylaxis: A prospective, observational safety study

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Abstract

Background Guidelines advocate >7 days of broad-spectrum antibiotics for unexplained fever (UF) during neutropenia. However, effective antimicrobial prophylaxis reduces the incidence of gram-negative infections, which may allow shorter treatment. This study evaluates the safety of discontinuing empirical broad-spectrum antibiotics if no bacterial source is documented after an initial work-up of 72 hours.

Methods We performed a prospective observational study at a tertiary-care hematology unit in patients suffering from hematological malignancies and treatment-induced prolonged neutropenia of ≥10 days. Oral fluoroquinolone and fluconazole prophylaxis was given from day 1. Fever was empirically treated with imipenem, which was discontinued after 72 hours if, following a standardized protocol, no bacterial etiology was documented. Duration of fever, antimicrobial therapy and overall mortality were registered.

Results A total of 166 patients were evaluated during 276 neutropenic periods. A total of 137 patients (82.5%) experienced ≥1 febrile episode. A total of 317 febrile episodes were observed, of which 177 (56%) were diagnosed as UF. In 135 febrile episodes (43%), a probable/definite infectious origin was documented. The mean duration of fever in neutropenic periods with 1 febrile episode was 5.5 days, and the mean time of treatment with imipenem was 4.7 days. In patients without documented infection, the mean time of imipenem treatment was only 3.7 days. Overall mortality 30 days after neutrophil recovery was 3.6% (6 of 166 patients); no patient died from untreated bacterial infection.

Conclusions Discontinuation of broad-spectrum antibiotics started for fever during neutropenia in hematology patients on fluoroquinolone and fluconazole prophylaxis is safe, provided that no bacterial etiology is documented after 72 hours.

Introduction

Severe and possibly life-threatening bacterial and fungal infections constitute a major cause of morbidity and mortality in patients with hematological malignancies treated with intensive chemotherapy, especially when the neutrophil count drops below 500 cells/µl for ≥10 days.[1-5] In case of fever, current guidelines universally recommend immediate empirical treatment with broad-spectrum antibiotics for at least 7 but even up to 14 days.[6] This approach is based on the assumption that most febrile episodes in these patients are due to bacterial infections. However, there is no evidence from randomized clinical trials that neutropenic patients with unexplained fever (UF), in whom evidence of a bacterial infection is lacking after a thorough diagnostic work-up, will benefit from prolonged antimicrobial therapy.

Although gram-positive bacteria account for the majority of all microbiologically documented infections, the more fulminate gram-negative infections result in a much higher attributable mortality.[3,5,7,8] The prophylactic administration of antibiotics, especially fluoroquinolones, has been demonstrated to result in a marked reduction of bacteremia due to gram-negative microorganisms,[9-13] and to reduce mortality,[14] probably by lowering the intestinal burden of these microorganisms. The argumentation in current literature that prolonged empiric broad-spectrum antibiotic therapy in case of UF during neutropenia will prevent recurrence of bacteremia and other bacterial infections does not take into account the efficacy of the administration of such antibiotic prophylaxis.

Moreover, apart from substantial extra costs and an increased amount of adverse events, continuing unnecessary treatment harbors additional disadvantage of antibiotic resistance selection. Also, it may falsely temper the scrutiny in the search for the real cause of the febrile episode. In fact, a significant proportion of these patients suffer from invasive fungal infections, especially invasive pulmonary aspergillosis (IPA), during the course of continuous neutropenic fever.[3]

The antibiotic strategy in case of prolonged neutropenia developed in our hospital over the last 10 years differs from most international recommendations. It is based on continuous fluoroquinolone and fluconazole prophylaxis, in combination with non-absorbable antibiotics for the first 10 days. In case of fever during neutropenia, patients are treated with a considerably shorter course of empirically started broad-spectrum antibiotics. This has been suggested as an alternative approach previously,[15,16] but the safety of such a strategy clearly needs to be evaluated in more detail before it can be widely accepted.

In our hospital, patients with prolonged neutropenia due to intensive chemotherapy or stem cell transplantation receive empirical treatment with imipenem when neutro-

penic fever is documented.[6,17] Imipenem is discontinued irrespective of the temperature response if, after a standardized diagnostic work-up of 72 hours, no bacterial etiology is identified. In case of a documented infection, patients receive directed therapy based on the susceptibility pattern of the identified pathogen (if available).

The primary objective of this prospective observational study was to investigate the safety of early discontinuation of empirically started broad-spectrum antibiotics for fever in neutropenic hematology patients during fluoroquinolone and fluconazole prophylaxis. As a secondary goal, we registered the outcome and duration of all febrile episodes within each neutropenic period.

Materials and methods

Patient population

The study was performed at the Erasmus Medical Center, a university tertiary care hospital in Rotterdam, The Netherlands. From March 2005 through June 2008, all consecutive adult hematology patients (age, >17 years) with expected duration of neutropenia (neutrophil count, <500 cells/μl) of ≥10 days, induced by intensive chemotherapy or hematological stem cell transplantation were studied. Excluded were patients with a carbapenem allergy. Patients had direct bloodstream access by means of a tunnelled central venous catheter (CVC), and were nursed in rooms with high-efficiency particulate air (HEPA) filtration. All patients gave written informed consent and the institutional review board approved the protocol.

Antimicrobial prophylaxis

All patients were on antimicrobial prophylaxis, which was started 1 to 5 days before chemotherapy or conditioning in case of stem cell transplantation, until neutrophil recovery was observed (neutrophil count, ≥200 cells/μl on 2 consecutive days). The standard oral regimen consisted of ciprofloxacin 500 mg BID and fluconazole 400 mg once daily. For the first 10 days of neutropenia, we added also colistin capsules 200 mg QID combined with 5 mg oral colistin suspension QID to accelerate the eradication of gram-negative bacteria and to minimize the theoretical risk of developing fluoroquinolone resistance in case of ciprofloxacin monotherapy. Prophylaxis was only given parenterally when oral intake was impaired (e.g., severe mucositis). Patients already initially at high risk for mucositis (e.g., in case of treatment with high-dose cytarabine, etoposide or methotrexate) also received a 10-day course of intravenous benzyl-penicillin from day 5 after the start of chemotherapy or longer in case of persisting mucositis, aimed to reduce the occurrence of bacteremia with oral streptococci. Bowel surveillance cultures consisting of mouth wash and rectal and vagina swab were taken twice weekly. If surveillance

cultures revealed ciprofloxacin-resistant gram-negative bacteria, ciprofloxacin was preferably replaced by sulphamethoxazole-trimethoprim and tobramycin was added or colistin was re-introduced, based on the susceptibility pattern, to aim for successful bowel decontamination.

Diagnostic and therapeutic protocol for neutropenic fever

Fever was defined as an ear temperature of >38.2°C during 2 subsequent occasions 1 hour apart, or a single measurement of >38.7°C. Disappearance of fever was defined as a temperature of <37.8°C for at least 24 hours following a febrile episode.

At the onset of fever, treatment with imipenem 500 mg QID was initiated, and a physical examination and microbiological work-up to detect any sign of clinically or microbiologically documented infection was performed. Signs of infectious sources that were specifically documented were oropharyngeal mucositis, erythema and/or tenderness or purulence of the insertion site or tunnel tract of the CVC, tenderness in the peri-anal region and signs of respiratory tract infection, including standard chest X-ray and imaging of sinuses if indicated. Microbiologically documented infection was defined as a microbiological investigation yielding a positive culture from blood using the Bactec system (BD; USA), preferably incubating an amount of 10 ml both aerobically and anaerobically), urine (except for concentrations $<10^3$/ml enterococci, coagulase-negative staphylococci (CNS), *Corynebacterium* spp. or *Candida* spp.), sputum or other respiratory secretions (except for normal pharyngeal flora), or material obtained by puncture. Definitions of catheter-related bloodstream infection (CRBSI) from current guidelines were followed in case of suspected CRBSI.[18] For this purpose, CVC blood cultures, together with peripheral blood cultures were taken for any new episode of fever. CRBSI was defined as ≥1 positive blood culture obtained from either CVC or peripheral vein (in case of CNS or other skin colonizers ≥2 CVC cultures were required) in patients with fever or other clinical manifestations of infection, lacking apparent other sources for infection except the catheter, combined with a culture of the catheter tip demonstrating the same phenotypic microorganism in case of catheter removal, or differential time-to-positivity (DTTP) of ≥2 hours of the peripheral minus the CVC blood culture.

If this initial diagnostic work-up did not reveal a clinically or a microbiologically documented bacterial infection, treatment with imipenem was stopped after 72 hours, regardless of the presence or absence of fever. If a causative pathogen was identified or a clinical source of infection was documented, therapy was tailored according to the susceptibility pattern or clinically documented focus. In case of bacteremia, tailored treatment was continued for 14 days, or at least 7 to 10 days in case of recovery from neutropenia.

Prolonged treatment with imipenem occurred in pre-defined specific occasions. Imipenem was continued in patients with clinical documented pneumonia or sinusitis

without a microbiologically documented pathogen. It was also continued in patients with fever and known unsuccessful gram-negative bowel decontamination. Patients with symptoms of septic shock (i.e., systolic blood pressure <90 mm Hg despite intravenous colloid administration and/or oliguria (<0.5 ml/kg/hr)) were treated with imipenem and amikacin 15 mg/kg for 1 to 3 days; in case of septic shock among patients with severe mucositis, intravenous vancomycin was added to assure adequate treatment in case of penicillin-resistant streptococci.

At day 5 of ongoing neutropenic fever, blood cultures for *Candida* spp. (Bactec Mycosis IC/F; BD) were taken and patients underwent high-resolution CT of the thorax, which was repeated 5 to 7 days later if fever persisted. In case of documented intrapulmonary abnormalities, patients underwent bronchoscopically guided bronchoalveolar lavage (BAL) of the most representative lung lesion. BAL fluid was cultured for bacteria, mycobacteria and fungi. Also, galactomannan level in BAL fluid specimens was measured to detect IPA. If bronchoscopy was not feasible because of the location or very small size of the lung lesion, galactomannan level was measured in serum specimens. If these diagnostic procedures were inconclusive, a biopsy of the lung lesions was performed, if feasible and not contraindicated. Patients were diagnosed with IPA following updated European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) definitions.[19] The presence of ongoing neutropenic fever without any abnormalities on high-resolution CT was not considered as an indication for empirical antifungal therapy.

Data collection and patient evaluation

Per patient data were collected for all consecutive neutropenic periods. Baseline information included age, sex, underlying hematological malignancy, type of stem cell transplantation and co-morbidity. Cases were evaluated by L.S. and B.J.R., who were not involved in patient management. For each patient, the outcome and mean duration of all febrile episodes within each neutropenic period were registered, together with the mean duration of imipenem use. All-cause mortality during neutropenia or within 30 days after neutrophil recovery was registered as a primary marker to determine the safety of our neutropenic fever treatment policy. Data were analyzed by means of descriptive statistics, using SPSS, version 15.0 (Chicago; Il).

Results

We included 166 patients receiving chemotherapy with an expected neutropenia duration of ≥10 days. In total, these patients experienced 276 periods of prolonged neutropenia. Characteristics of the patient population are presented in table 1, and

Table 1. Characteristics of 166 hematology patients with prolonged neutropenia.

Age, mean years (range)	53.9 (19-80)
Male sex	98(59.0)
Underlying hematological disorder	
AML-MDS (137 neutropenic periods)	64 (38.6)
Multiple myeloma (47 neutropenic periods)	47 (28.3)
Non-Hodgkin lymphoma (64 neutropenic periods)	36 (21.7)
Acute lymphatic leukemia (16 neutropenic periods)	8 (4.8)
Other[a] (12 neutropenic periods)	11 (6.6)
Co-morbidity	
Cardiovascular	42 (25.3)
Respiratory	7 (4.2)
Other[b]	5 (3.0)

Note. Data presented are numbers (%) of patients unless otherwise indicated. AML-MDS, acute myeloid leukemia-myelodysplastic syndrome.
[a]Hodgkin lymphoma (n=6), chronic myeloid leukemia (n=3), aplastic anemia (n=2). [b]Autoimmune disorders requiring treatment with immunosuppressive therapy (n=3), ulcerative colitis (n=1), HIV (n=1).

characteristics of these 276 neutropenic periods are shown in table 2. A total of 182 (65.9%) of these neutropenic periods were related to high-dose chemotherapy, and 94 were in the context of hematological stem cell transplantation. The mean duration of neutropenia was 20.5 days. The longest mean neutropenic period (28.2 days) was observed in patients with acute myeloid leukemia-myelodysplastic syndrome (AML-MDS) treated with remission and/or consolidation chemotherapy.

Table 2. Main characteristics of the neutropenic[a] periods (n=276).

Hematological treatment course (%)	
High-dose chemotherapy	182 (65.9)
Autologous stem cell transplantation	86 (31.2)
Allogeneic stem cell transplantation	8 (2.9)
Mean days of neutropenic period (SD)	
All neutropenic periods	20.5 (11.5)
AML-MDS (remission and consolidation, n=127)	28.2 (11.3)
MM, autologous stem cell transplantation (n=47)	12.7 (4.0)
Non-Hodgkin lymphoma (n=64)	14.6 (6.7)
Acute lymphatic leukemia (n=16)	18.3 (11.7)
Allogeneic stem cell transplantation (n=8)	11.9 (1.7)
Neutropenic periods classified according to *febrile* episodes	
Neutropenia without fever	74
Neutropenia with 1 febrile episode	113
Neutropenia with 2 febrile episodes	57
Neutropenia with >2 febrile episodes	32

Note. Data presented are numbers of neutropenic periods. Neutropenia, neutrophil count, <500 cells/µl; SD, standard deviation; AML-MDS, acute myeloid leukemia-myelodysplastic syndrome; MM, multiple myeloma.

Of all 166 patients, 29 (17.5%, accounting for 39 neutropenic periods) did not develop fever during their entire hematological treatment. A total of 137 patients (82.5%) experienced 1 or more febrile episodes in the remaining 202 periods of neutropenia (table 2). Mean duration of all-cause fever in neutropenic periods with only 1 febrile episode (n=113) was 5.5 days, and in these patients imipenem was given for a mean duration of 4.7 days (SD, 4.3 days). A clinical or a microbiological infection was documented in 48 of these episodes; 64 episodes were diagnosed as UF, and in 1 case a non-infectious origin of fever was registered. If only taking into account the 64 episodes of UF, the observed duration of fever was 4.3 days (SD, 3.6 days), and imipenem was administered for 3.7 days (SD, 1.8 days). For neutropenic periods with 2 febrile episodes, the mean total duration of fever was 9.9 days, and the total duration of imipenem use was 6.6 days (SD, 4.0 days). Finally, for periods of neutropenia with >2 febrile episodes, the mean total duration of fever was 16.8 days, and total imipenem use 10.5 days (SD, 6.0 days).

Of all 276 neutropenic periods, 74 passed without fever. In the remaining periods, treatment with imipenem was started on 317 occasions of fever (figure 1). In 169 episodes, imipenem was discontinued after 72 hours as the diagnostic work-up revealed no bacterial etiology (n=155). Directed therapy, based on microbiological culture results or clinically documented infection, was started on 92 occasions of fever. In the majority of all cases in which tailored therapy was administered, a switch to a glycopeptide, mainly vancomycin, was made because of gram-positive infections. Prolonged treatment with imipenem was given for 56 instances, including 22 episodes of UF in which patients were considered too ill to stop empirically started imipenem safely, despite the absence of any clue of a bacterial etiology. Other reasons to continue imipenem were clinically documented infection, especially pneumonia and typhlitis (neutropenic enterocolitis). In 18 patients, antifungal therapy was eventually started at the time a diagnosis of possible or probable/proven invasive fungal infection was made.

In figure 2, the presumed etiology of all 317 febrile episodes in the 202 neutropenic periods is provided. Distinct febrile episodes caused by the same etiology (e.g., IPA) during a single period of neutropenia were counted only once. The vast majority of all febrile episodes were diagnosed as UF (n=177). Episodes of primary bacteremia were due to CNS (n=21), *Enterococcus* spp. (n=2), combined CNS and *Enterococcus faecalis* (n=1), *Streptococcus* spp. (n=3), *Escherichia coli* (n=1), and *Rothia mucilaginosa* (n=1). In the patient with *Escherichia coli* bacteremia, surveillance cultures did not detect gram-negative microorganisms before the onset of bacteremia.

Sources of secondary bacteremia were CRBSI (n=13), bacteremia in the context of severe mucositis and/or typhlitis (n=11), respiratory tract infection (n=4), cellulitis (n=1), diverticulitis (n=1), and an infected venous thrombus (n=1). Infections without bacteremia were catheter insertion site infection (n=12), IPA (n=18, of which 7 probable/

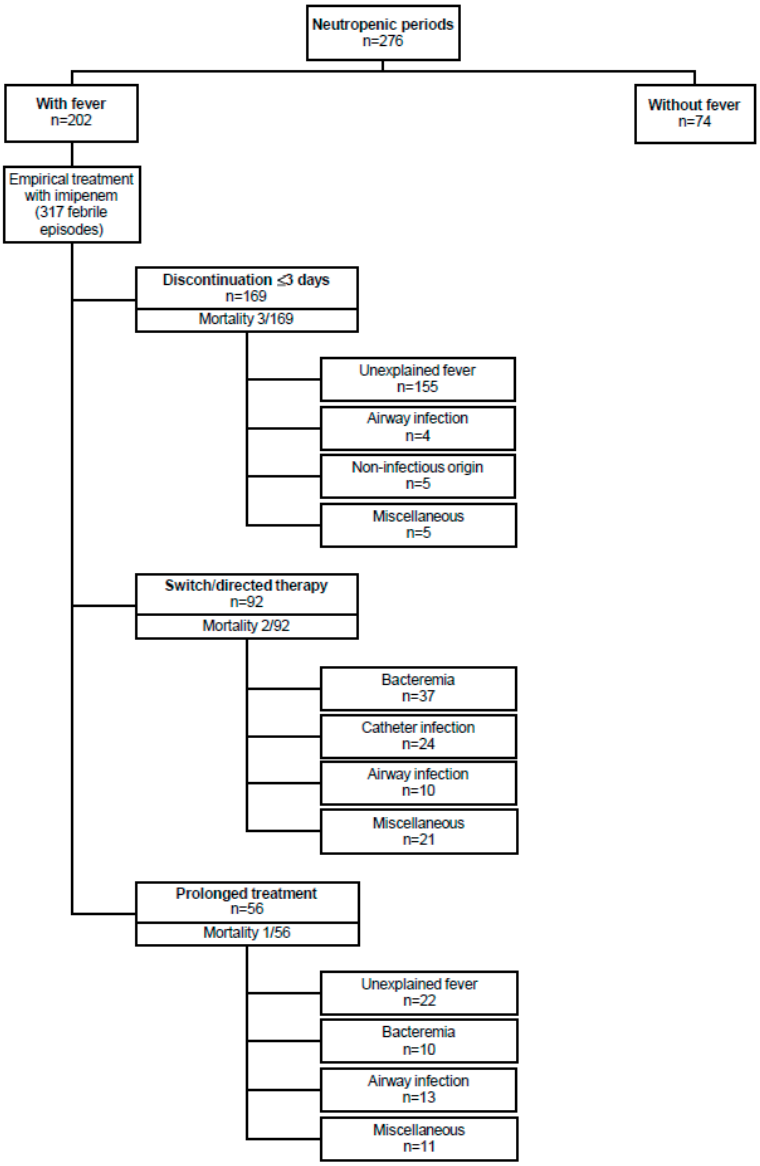

Figure 1. Antibiotic strategy in 276 neutropenic periods (317 febrile episodes).

proven and 11 possible), typhlitis (n=4), viral infection (n=5), urinary tract infection (n=2), wound infection (n=2), and tonsillitis, folliculitis, meningitis, *Rhodotorula* pleuritis, and disseminated mucormycosis (all, n=1). In 5 episodes of fever, a non-infectious etiology was the most likely explanation, i.e., fever due to blood transfusion (n=3), exacerbation of gout, and acute graft-versus-host disease (both, n=1).

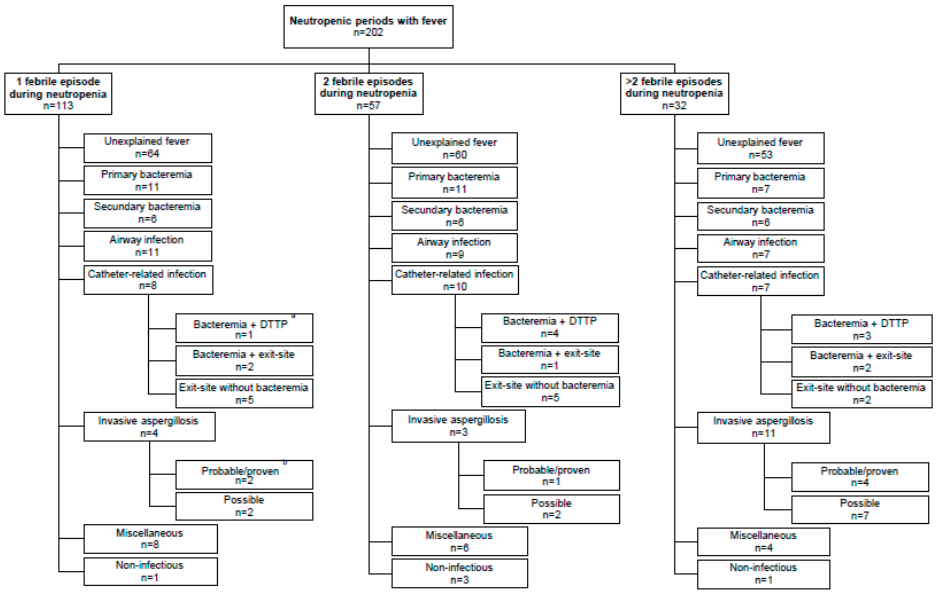

Figure 2. Cause of fever in 202 neutropenic periods (137 patients). Febrile episodes due to the same etiology during a single neutropenic period were counted once; therefore, adding up totals may result in different numbers compared to the multiplication of the number of neutropenic periods with the febrile episodes.
[a]DTTP, differential time-to-positivity.

Six of the 166 patients (3.6%) died within 30 days after neutrophil recovery; 4 patients died while being neutropenic, and 2 patients had already recovered from neutropenia (table 3). No differences in outcome were observed when taking into account the original decision to stop or to continue empirical treatment with imipenem (figure 1). Infectious complications contributed to death in 2 patients (IPA and severe typhlitis). The cause of death in the other patients was cardiac death after electromechanical dissociation (autopsy refused), cardiogenic shock due to myositis with left ventricular dysfunction, acute respiratory distress syndrome (no documented infectious origin in BAL), and progressive AML-MDS (all, n=1).

Discussion

Current guidelines recommend prolonged use of broad-spectrum antibiotics in febrile patients with prolonged neutropenia, although also emphasizing that these recommendations are general and should be applied taking into account the different treatment settings, patient populations and antimicrobial susceptibility patterns.[6] Even in patients already non-febrile after 3 to 5 days of treatment, cautious recommendations are to continue antibiotics in these high-risk patients for at least 2 weeks.

Table 3. Data on fatal outcome of 6 patients.

Infectious cause	Underlying disease	Cause of death	Days after onset of neutropenia
Yes	AML-MDS	Proven/probable aspergillosis jaw and lung	36
Yes	AML-MDS	Refractory AML, typhlitis and possible IPA	48
No	AML-MDS	ARDS, no infectious origin	41*
No	AML-MDS	Reanimation after EMD, no autopsy	15
No	AML-MDS	Progressive AML	28
No	HL	Cardiogenic shock due to left ventricular dysfunction induced by myositis	16*

Note. *Patients already recovered from neutropenia. AML-MDS, acute myeloid leukemia-myelodysplastic syndrome; HL, Hodgkin lymphoma; ARDS, acute respiratory distress syndrome; EMD, electromechanical dissociation; neutropenia, neutrophil count, <500 cells/µl.

Indeed, progression of infection in patients with prolonged neutropenia can be rapid, and signs and symptoms of inflammation may be minimal or even absent. However, we are unaware of any convincing evidence from randomized clinical trials that non-febrile or even febrile patients, in whom proof of a bacterial infection is lacking after a scrupulous clinical and diagnostic work-up, will benefit from continued antimicrobial therapy. A considerable amount of febrile episodes in patients with chemotherapy-induced neutropenia have a non-infectious etiology. Neutropenic hematology patients might be prone to febrile reactions in general, due to the nature and consequences of treatment with high-dose chemotherapy and transfusions with blood or plasma products. Moreover, a significant proportion of these patients are diagnosed with invasive fungal infections, especially IPA, in case of persisting neutropenic fever. Continuation of broad-spectrum antibiotics may falsely temper the scrutiny in the search for the real cause of the febrile episode in such cases.

This study suggests that in patients receiving fluoroquinolone and fluconazole prophylaxis, broad-spectrum antimicrobial therapy started empirically for neutropenic fever can be safely discontinued after 72 hours if no infectious origin is documented, provided that patients are hemodynamically stable. Following this policy, the all-cause mortality in our study population was 3.6% (6 of 166 patients). At least 1 and possibly 2 of these deaths were related to a non-bacterial infection (IPA) and another one was due to refractory AML-MDS. In the 4 other patients, a bacterial etiology could not be established although autopsy was not performed.

The major advantage of a more restrictive antimicrobial treatment strategy would be an important reduction in the amount of days on which patients receive broad-spectrum antibiotics. Following latest Infectious Disease Society of America- guidelines for these high-risk patients would imply an additional prescription of at least another 5 to 7 days of broad-spectrum antibiotics after defervescence. This study suggests that fluoroqui-

nolone prophylaxis effectively prevents infections with gram-negative microorganisms. In only 1 patient a gram-negative bacteremia was documented. However, no gram-negative microorganisms were detected in preceding surveillance cultures in this particular patient. In contrast, the majority of bacterial infections were due to gram-positive microorganisms, and microbiological confirmation of gram-positive bacteremia was straightforward in these cases. Therefore, early discontinuation of empirically started broad-spectrum antibiotics could be done safely in the absence of a bacterial origin after a thorough diagnostic work-up. Although disadvantages of antibiotic prophylaxis are conceivable, the results of a recent meta-analysis on antibiotic prophylaxis during chemotherapy induced neutropenia demonstrated that the reduction in mortality and infection rates outweighs the risk of developing resistance, costs, and adverse events.[14]

The present study has several limitations that should be noticed. This study lacked a control arm because the strategy as described has become the present standard of care in our hospital. Further studies should therefore compare this policy with the more widely advocated approach of continued broad-spectrum antibiotic therapy for another 7 days after defervescence. Also, it should be stressed that effective antimicrobial prophylaxis, which was monitored twice weekly by taking surveillance cultures, is an essential part of our strategy.

From a historical point of view, the management of UF during neutropenia has been an evolving landscape over the previous 20 years. Not even 10 years ago, vancomycin was part of the empiric therapy in many centers, but subsequent randomized trials showed that vancomycin-containing regimens did not lead to a better outcome.[20] More recently, the need for empirical antifungal therapy in case of UF of >5 days has been questioned due to the improved outcome with voriconazole treatment, as well as the possibility to diagnose invasive aspergillosis earlier by means of new diagnostic tools, such as galactomannan measurement and high-resolution CT of the lungs. These developments improved the outcome of invasive aspergillosis considerably.[21-24] In this historical perspective, our present study could be a cautious next step in this evolving field on the management of neutropenic fever.

In conclusion, our study shows that discontinuation of empirically started broad-spectrum antibiotics for fever during neutropenia in hematology patients on continuous fluoroquinolone and fluconazole prophylaxis is safe, provided that no bacterial origin is documented after 72 hours.

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Part III

Catheter-related bloodstream infection

Chapter 6

Comparison of the roll plate method to the sonication method to diagnose catheter colonization and bacteremia in patients with long-term tunnelled catheters: A randomized, prospective study

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Abstract

Background Diagnosing catheter-related bloodstream infection (CRBSI) still often involves catheter tip culture. The conventional method is the semi-quantitative roll plate method. However, the use of the quantitative sonication technique could have additional value, as it may detect endoluminal microorganisms more easily. Because endoluminal infection tends to occur in long-term central venous catheters, we compared both techniques for patients with long-term tunnelled catheters.

Methods For 313 consecutive central venous catheters tips from 279 hematology patients, colonization detection rates were compared by performing both techniques in a random order, using conventional detection cut-offs. Additionally, for the subgroup of patients with clinical suspicion of CRBSI (n=89), the diagnostic values of both techniques were compared.

Results The overall tip colonization rate was 25%. For each technique, the detection rate tended to be better if that technique was performed first. The diagnostic performance for the subgroup of patients with clinical suspicion of CRBSI was limited and not different for both methods. Sensitivity and specificity were 45% and 84%, respectively, for the sonication technique versus 35% and 90%, respectively, for the roll plate method. The fact that 35 of 40 patients with CRBSI received antimicrobial therapy before catheter removal and tip culture, in an attempt to salvage the catheter, may partly explain this poor performance. No differences were observed when catheters were stratified according to in situ time below or above the median of 4 weeks.

Conclusions The sonication culture technique was not better than the roll plate method to diagnose tip colonization or CRBSI in patients with long-term tunnelled catheters.

Introduction

Catheter-related bloodstream infection (CRBSI) is still one of the leading causes of nosocomially acquired bacteremia, with significant contributions to morbidity, costs and to a lesser extent, also mortality.[1-5] Although ideally the diagnosis of CRBSI is made before catheter removal, a definite diagnosis still often involves a culture from the catheter tip.[6,7] The reference standard of tip culture is a semi-quantitative technique, described by Maki et al. in 1977, with a cut-off of 15 colony forming units (cfu) to distinguish microbial contamination of catheters from significant colonization.[8] This technique is also called the roll plate method, as the catheter tip is rolled back and forth on an agar plate for culture. However, because catheter colonization and infection can be the consequence of the introduction of intraluminal microorganisms during manipulation of the catheter hubs or the infusion of fluids or drugs, this technique may be less appropriate for the detection of endoluminal tip colonization.

Other methods were developed subsequently, in an attempt to deal with this and other limitations.[9-15] One promising method with the claimed ability to detect both endoluminal and exoluminal microorganisms is the quantitative sonication technique, first described by Constantinou et al.,[16] and validated later. In early years, most studies were performed using cut-offs for catheter tip colonization of ≥1000 cfu/catheter segment for quantitative techniques, although lower breakpoints were also used.[17] Nowadays, laboratory criteria usually accept a breakpoint of ≥100 cfu/catheter segment, which is also recommended in current Infectious Diseases Society of America guidelines.[6,18] Recently, Bouza and colleagues demonstrated a cut-off of ≥100 cfu to be superior to one of ≥1000 cfu/catheter segment.[19]

The number of prospective studies that compare the semi-quantitative and quantitative techniques, however, is limited.[12,18-22] These studies report conflicting results, and are non-homogenous with respect to the type of catheter studied or the length of time that devices remained in place, two important factors that determine the risk of CRBSI.[2,23] Because endoluminal contamination is thought to be the most frequent route of microbial colonization in patients with catheters with a long dwell time, quantitative methods may be especially appropriate for this patient category.[6,7,23,24] However, no comparative prospective studies have been performed with this subgroup, and no gold standard exists.

The present study describes the results of a prospective, randomized study to compare the yields of both techniques to detect catheter tip colonization in patients with long-term tunnelled catheters. Tip colonization is a relevant endpoint, as the incidence of tip colonization was demonstrated to correlate well with the incidence of CRBSI in a recent meta-analysis.[25] We also assessed whether performing tip culture, especially by

the sonication technique, for patients with clinical suspicion of CRBSI could give additional diagnostic information to rule out or establish CRBSI.

Materials and methods

Patient population

The study was performed at Erasmus Medical Center, a university referral hospital in Rotterdam, The Netherlands. All consecutive tunnelled central venous catheters (CVCs) were derived from adult patients (age, >17 years) with hematological disease, mainly patients with acute myeloid leukemia or myelodysplastic syndrome. Some patients were treated with allogeneic stem cell transplantation and patients invariably suffered from prolonged neutropenia due to the anti-leukemic treatment. Catheters removed for any reason (e.g., end of therapy or suspicion of CRBSI) in the period from April 2005 through December 2007 were sent to the Department of Microbiology for tip culture. No antimicrobial-coated catheters were used, and connection to the infusion system was established through the use of a needle-free closed connector valve system (Bionector; Vygon). All catheters had been placed in the radiology room under full sterile barrier precautions, and were mainly used for the administration of intravenous medication and chemotherapy. Data were collected on the use of antimicrobials before catheter removal, catheter dwell time, and reason for removal.

Microbiological procedures

Catheter tips were processed using the conventional roll plate method and the sonication technique in a random order. Randomization occurred at the microbiology laboratory. The semi-quantitative method of Maki et al. was performed by rolling the external surface of a catheter tip back and forth on the surface of a Columbia agar plate supplemented with 5% sheep blood (Bactec; BD) at least three times and then incubating the plate for 72 hours at 5% CO_2 and 35°C, after which the numbers of cfu were quantitated as described in detail elsewhere.[8] The sonication technique was performed by placing the catheter in 5 ml of 0.9% NaCl, sonication for one minute (Soniprep 150 instrument with a 23-kHz generator; MSE Ltd.), and vortexing it for 15 seconds. Fifty µl of the sonication fluid was cultured on Columbia agar, allowing for a detection limit of ≥100 cfu/catheter tip. Finally, the tip was incubated in tryptic soy agar broth. If growth of 1-3 cfu was observed on the agar plate on which the sonication fluid had been inoculated, the identification of these colonies was confirmed by broth culture of the tip to exclude contamination of the plate. Microorganisms recovered from the plates were identified and counted by standard microbiological methods. Blood cultures were processed according to routine procedures (Bactec; BD).

Definitions

Catheter tip colonization was defined as a positive semi-quantitative tip culture of ≥15 cfu/ml for the roll plate method or ≥100 cfu/catheter segment for the sonication technique, as described elsewhere.[8,9,18,19]

Definitions of CRBSI and catheter colonization from current guidelines were followed.[6,26,27] CRBSI was defined as 1 or more positive blood cultures (at least 2 blood cultures for coagulase-negative staphylococci) obtained from a peripheral vein for patients with clinical manifestations of infection and no apparent other source of infection except for the catheter AND a catheter tip culture with the same phenotypic microorganisms OR differential time-to-positivity (DTTP) of ≥2 hours for the peripheral minus the CVC blood culture. Endoluminal CRBSI was defined as a positive hub culture AND DTTP of ≥2 hours OR a positive hub culture with the presence of the same microorganism both in peripheral blood and on the catheter tip in the absence of insertion site infection and in the absence of any other source of infection. Exoluminal CRB was defined as clinical signs of an insertion site or tunnel infection combined with a negative hub culture, but with either DTTP of ≥2 hours OR positive blood and catheter tip cultures for the same phenotypic microorganism.

Data-analysis

The presence of significant counts of microorganisms assessed by any of the two techniques, using the cut-off values as described above, was considered the reference standard for detection. Proportions of detection of tip colonization were calculated for both techniques, taking into account the randomly assigned order.

For the subgroup of patients with clinical suspicion of CRBSI and/or insertion site infection with concomitant bacteremia, the sensitivity, specificity, and predictive values with corresponding 95% confidence intervals (Wilson score interval method with continuity correction) were calculated for both techniques separately and combining the results of the two culture methods. In a further exploratory analysis, catheters were stratified according to dwell time (below or above the median dwell time), and sensitivity, specificity, and predictive values were calculated for both techniques. Statistical analyses were performed using SPSS, version 13.0 (Chicago; Il) and VassarStats (New York; NY).

Results

A total of 313 catheter tips from 279 patients were analysed. The mean dwell time was 55 days (range, 4-469 days). Colonization was detected in 77 of 313 catheter tips (25%). Data are presented in figure 1. Data were also analysed with catheters stratified according to the procedure order. For 159 tips, the roll plate method was performed

first, whereas for the other 154 catheter tips the sonication technique was performed first. In the sample in which the roll plate method was performed first, tip culture was positive in 38 of 159 cases (24%) with the roll plate method and in 23 of 159 cases (14%) with the sonication technique. In the sample of 154 catheters in which the sonication technique was performed first, the tip culture was positive in 30 cases (19%) detected by the sonication technique and in 28 cases (18%) detected by the roll plate method.

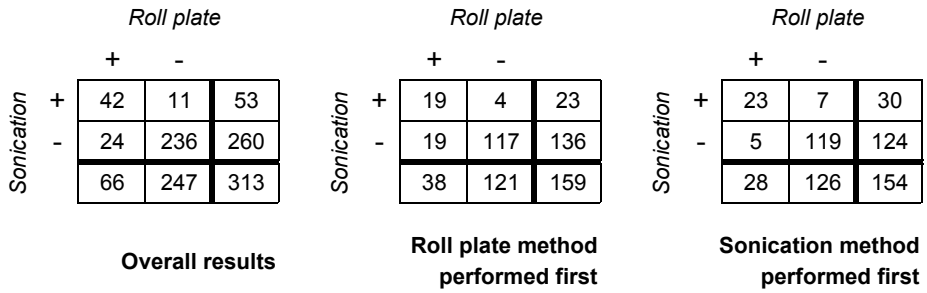

Figure 1. Detection of catheter tip colonization in 313 tunnelled catheters. Data are presented as overall numbers together with results stratified according to procedure order. Cut-offs used for detection of colonization were ≥15 colony forming units the roll plate method and ≥100 colony forming units/catheter segment for sonication.

A total of 89 catheters were removed because of clinical suspicion of CRBSI and/or insertion site infection with concomitant bacteremia. The mean dwell time for this subgroup was 56 days (range, 4-447 days). CRBSI in agreement with the aforementioned definitions was eventually diagnosed in 40 of these 89 catheter episodes (36 patients). Of these episodes, 7 were in agreement with the definition of an insertion site CRBSI, and in another 6 episodes, an endoluminal CRBSI was diagnosed. For the remaining 27 episodes of CRBSI, the distinction could not be made, which means that in these cases the hub cultures were negative and no signs of insertion site infection were present but DTTP of ≥2 hours was recorded, or the result of a positive peripheral blood culture was concordant with the catheter tip culture. In 35 of the 40 episodes of CRBSI, antibiotic therapy with activity against the isolated microorganism had been administered in an attempt to salvage the catheter before the catheter tip was eventually cultured.

For this subgroup of 89 catheters with clinical suspicion of catheter-related infection, the diagnostic yields and predictive values were calculated for both techniques separately and in combination (table 1). The sensitivity was disappointingly low for both catheter tip culture methods. In contrast, for both techniques the specificity and positive predictive values were better.

Table 1. Diagnostic parameters for both tip culture techniques applied to catheters removed for suspected CRBSI or insertion site infection with bacteremia (n=89).

	Sonication	Roll plate	Combined data
Sensitivity	45 (30-61)	35 (21-52)	48 (32-64)
Specificity	84 (70-92)	90 (77-96)	84 (70-92)
Positive predictive value	69 (48-85)	74 (49-90)	70 (50-86)
Negative predictive value	65 (52-76)	63 (50-74)	63 (50-74)

Note. Data are % (95% confidence-intervals). Cut-offs for detection of tip colonization were ≥15 colony forming units for the roll plate method and ≥100 colony forming units/catheter segment for sonication.

Finally, we stratified these 89 catheters according to dwell time, creating groups with the median value (28 days) as a cut-off, to compare results for long-term and very long-term catheters. Sensitivity, specificity, and predictive values for these subgroups were not different from those for the complete set of 89 catheters (data not shown).

Discussion

In this study, we demonstrated that the use of the quantitative sonication technique to detect catheter tip colonization in patients with long-term tunnelled CVCs had no surplus value compared with the semi-quantitative roll plate method. In addition, the diagnostic value of a catheter tip culture for patients with a tunnelled catheter under clinical suspicion of having a CRBSI seems limited, regardless of the method used. For both techniques, the diagnostic yield was lower if a culture technique was performed after the other one then if it was performed first. This is partly in accordance with the observation made by Sherertz and colleagues,[18] who observed the same for the sonication technique.

The fact that 35 of the 40 patients with CRBSI received antimicrobial treatment prior to catheter tip culture is a likely explanation for the observed low sensitivity of tip culture. Antibiotic therapy in an attempt to salvage the catheter will almost inevitably be given to patients with suspected CRBSI from a tunnelled catheter. It is conceivable that this lowers the culture yield, both from the outer surface and from the endoluminal surface. Because these antimicrobial agents are administered through the CVC, endoluminal microorganisms are exposed to much higher antibiotic concentrations than are exoluminal bacteria. Antimicrobial pre-treatment may therefore influence the sensitivity of the sonication technique in particular. The negative impact of antimicrobial pre-treatment on the diagnostic yield of catheter tip culture was recently demonstrated for short-term catheters.[28]

Other explanations for why the sonication technique did not perform better than the roll plate method are possible. In this study, all catheters were equipped with a disinfectable needle-free closed connector system. If used properly, this may decrease the risk of endoluminal CRBSI.[29] Finally, the sonication technique may not be able to remove microorganisms from the endoluminal biofilm sufficiently.

The practice of pre-treatment with antibiotics does raise the question of whether using lower cut-off values for colonization detection might be beneficial. In a separate analysis applied to patients with clinical suspicion of CRBSI, data obtained with the method described by Maki et al. were recalculated, using modified cut-offs for colonization, considering any growth of microorganisms on catheter tips concordant with the yield of blood cultures as a positive result. This did indeed improve the sensitivity of this method from 35 to 58%, at only a limited cost of specificity (86 instead of 90%). Future studies should be performed to investigate this observation more specifically before stating that lower cut-offs may be preferred for patients receiving antimicrobial therapy before tip culture. For the sonication technique, we did not evaluate the sensitivity of cut-offs below 100 cfu/catheter segment because this would have implied the inoculation of the total of 5 ml of sonication fluid on at least 10 agar plates, which we decided not to do because it would be very labor-intensive and therefore unacceptable in routine patient care.

To our knowledge, this is the first prospective, randomized study in which the conventional roll plate method was compared with the sonication technique for patients with long-term tunnelled CVCs. In earlier studies, both techniques were compared in other, non-homogenous patient populations with short-term devices.[12,18-22] In a recent study of 1000 *short-term* CVCs, Bouza et al. demonstrated the sonication technique (1 minute at 55,000 Hz and 125 W) to be less sensitive than the roll plate method.[19] For the roll plate method, a breakpoint of ≥15 cfu was used in this study, and for the sonication technique, cut-offs of both ≥100 and ≥1000 cfu/catheter segment were studied, of which ≥100 cfu/catheter segment demonstrated superiority for detection of tip colonization. However, for the subgroup of long-term catheters (defined as >6 days), the sensitivity of both methods was comparable.

Unequivocally, the hypothesis that the sonication technique could have additional diagnostic value due to its ability to detect endoluminal microorganisms is attractive. It has been suggested that the endoluminal route of catheter infection becomes dominant over the catheter insertion site as the source of infection in patients with long-term devices.[6,7,23,24] This may explain why the sonication technique gave slightly better, although non-significant, results than those by the roll plate method for the "long-term" subgroup of the study by Bouza et al. Taking into account that in this study long-term use was defined as >6 days suggests that these results could be even more pronounced

if truly long-term catheters are studied, as in this study. However, we were unable to confirm this hypothesis. In a sample of 313 CVCs and arterial catheters from a mixed patient population, Raad and colleagues found fairly better diagnostic parameters for the sonication technique (at 55,000 Hz and 125 W) than for the roll plate method.[20] For the roll plate method, cut-off levels of ≥15 cfu to ≥1000 cfu were studied, and for the sonication technique, breakpoints of $\geq 10^2$ until $\geq 10^4$ cfu were evaluated. Considering the results obtained by using the same breakpoints as those in our study, levels of sensitivity, specificity, and positive and negative predictive values for CRBSI were reported to be 78%, 88%, 35%, and 98%, respectively, by using the roll plate method, compared with 93%, 94%, 72%, and 99%, respectively, for the sonication technique. No details are given on statistical significance, and for the given values only one of both procedures was performed on a single catheter tip. Also, Sherertz et al. reported better sensitivity with the sonication technique then with the roll plate method (53% versus 33%; $P<.05$), using cut-offs of ≥15 cfu for the roll plate method and ≥100 cfu/catheter segment for the sonication technique, for intensive care unit patients.[18] Other researchers did not find differences in diagnostic performance between both techniques.[12,20-22]

According to current guidelines, routinely culturing the catheter tip is not recommended to avoid overtreatment of clinically insignificant tip colonization in patients without suspicion of CRBSI. Therefore, we determined sensitivity, specificity, and predictive values for both techniques for the subset of catheter episodes in which there was clinical suspicion of CRBSI. Establishing the diagnosis of CRBSI in these patients is preferably done by means of non-invasive diagnostic tests while the catheter is left in place. However, a reliable diagnostic test that can confirm or reject CRBSI in cases when the catheter is eventually removed would be helpful. The positive predictive value observed in this study could help to establish the diagnosis of CRBSI, but the low sensitivity does not allow the use of tip culture to reject the diagnosis of CRBSI.

In conclusion, for patients with long-term tunnelled CVCs, the diagnostic yields of the roll plate method and the sonication technique were comparable, although the sensitivities of both methods were low. This might be due to attempts to salvage the catheter by administering antibiotics in case of suspected CRBSI to most of these patients in the days before catheter removal and tip culture. With this respect, our observation that lowering conventional tip colonization cut-offs can improve diagnostic accuracy could be valuable.

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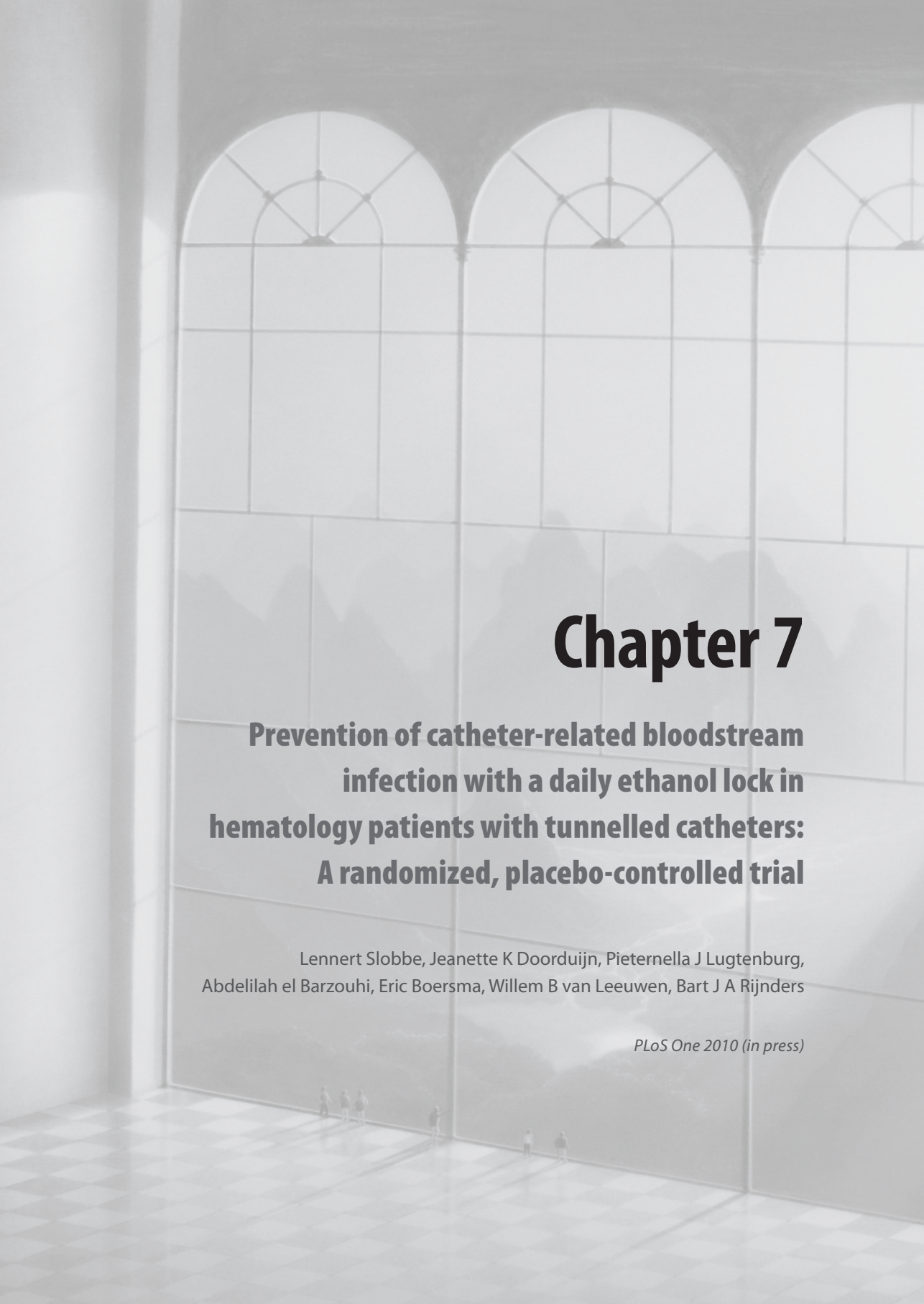

Chapter 7

Prevention of catheter-related bloodstream infection with a daily ethanol lock in hematology patients with tunnelled catheters: A randomized, placebo-controlled trial

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Abstract

Background Catheter-related bloodstream infection (CRBSI) results in significant attributable morbidity and mortality. In this randomized, double-blind, placebo-controlled trial, we studied the efficacy and safety of a daily ethanol lock for the prevention of CRBSI in patients with a tunnelled central venous catheter (CVC).

Methods From 2005 through 2008, each lumen of the CVC of adult hematology patients was locked for 15 minutes per day with either 70%-ethanol or placebo, where after the lock solution was flushed through. As a primary endpoint, the incidence rates of endoluminal CRBSI were compared.

Results The intent-to-treat analysis was based on 376 patients, accounting for 448 CVCs and 27,745 catheter days. For ethanol locks, the incidence of endoluminal CRBSI per 1000 CVC-days was 0.70 (95%-CI, 0.4-1.3), compared to 1.19 (95% confidence interval, 0.7-1.9) for placebo (incidence rate-ratio, 0.59; 95% confidence interval, 0.27-1.30; P=.19). For endoluminal CRBSI according to the strictest definition (positive hub culture and identical bacterial strain in blood), a 3.6-fold, non-significant, reduction was observed for patients receiving ethanol (2 of 226 versus 7 of 222; P=.103). No life-threatening adverse events were observed. More patients receiving ethanol discontinued lock-therapy (11 of 226 versus 1 of 222; P=.006) or continued with decreased lock-frequency (10 of 226 versus 0 of 222; P=.002), due to non-severe adverse events.

Conclusions In this study, the reduction in the incidence of endoluminal CRBSI using preventive ethanol locks was non-significant, although the low incidence of endoluminal CRBSI precludes definite conclusions. Therefore, the lack of statistical significance may partially reflect a lack of power. Additional studies should be performed in populations with higher incidence of (endoluminal) CRBSI. Alternative sources of bacteremia, like exoluminal CRBSI or microbial translocation during chemotherapy-induced mucositis may have been more important in our patients.

Introduction

The indwelling central venous catheter (CVC) has become an essential feature of modern patient management. However, its use puts patients at risk for various complications, especially catheter-related bloodstream infection (CRBSI). CRBSI accounts for a major cause of healthcare-related bacteremia and leads to prolonged hospital stay and significant attributable costs.[1-4] Reported attributable mortality varies from 2% up to 25% in critically ill patients.[2,5] In a meta-analysis, the odds-ratio for mortality in patients with CRBSI was 1.65 compared to control patients who were matched for severity of illness.[5]

In contrast to short-term CVCs, CRBSI in patients with tunnelled or implanted devices is thought to be mainly caused by endoluminal colonization due to contamination of the catheter hub.[6,7] Evidence-based recommendations on CRBSI prevention have been published.[8,9] To some extent, endoluminal CRBSI can be prevented if an *antibiotic* solution is instilled in the catheter.[10-12] However, the preventive use of antibiotics should be avoided if alternative options exist.[13,14] Although there is evidence to support the concept, methodologically appropriate clinical studies on the use of preventive *antiseptic* solutions are scarce. For this purpose, ethanol is increasingly considered as a promising candidate. For CRBSI-*treatment*, an ethanol-lock has been demonstrated to be efficacious in several observational studies.[15-17] More recently, an ethanol lock has also been studied for CRBSI-*prevention*.[18-21] A major advantage of ethanol would be the broad antimicrobial spectrum without compromising future antibiotic treatment. Furthermore, it is cheap and universally available.

In the current randomized, clinical trial, we study the efficacy and safety of a daily 70%-ethanol lock on the prevention of endoluminal CRBSI in hematology patients with long-term tunnelled catheters.

Materials and methods

The study was performed at the Erasmus Medical Center, a tertiary referral hospital with 2 locations in Rotterdam, The Netherlands. Eligible study-participants were all consecutive adult (age, >17 years) hematology patients with a tunnelled silicone CVC, inserted in the preceding 72 hours before study-entry. Excluded were patients with an alcohol-intolerance or concomitant treatment with metronidazole. Patients were enrolled from July 2005 through August 2008. The institutional review board approved the protocol; written informed consent was obtained from all patients.

Study design and ethanol lock procedure

The study was a randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov identifier: NCT00122642). Randomization was performed using a computer-generated list of randomly assigned permuted blocks. Randomization was catheter-based, which implies that patients could be randomized more than once if insertion of a new CVC was needed. Concealment of allocation and provision of blinding was guaranteed by uninvolved employees of the Department of Pharmacy, who delivered patient-labelled ampoules containing either 70%-ethanol or placebo (0.9% NaCl).

During hospitalization, every lumen of the CVC (3 ml) was locked for 15 minutes per day, following which the solution was flushed through with 10 ml 0.9% NaCl. During outpatient settings, the lock was instilled by the nursing staff once weekly before renewal of the regular heparin solution.

An investigator-blinded safety analysis was performed after inclusion of 80 patients, as some patients experienced adverse effects immediately after flushing the lock solution through. This provisional analysis led to an amendment that allowed these patients to continue with a modified lock regimen, in which only 1 lumen was locked per day.

Data collection and definitions

Baseline characteristics included age, sex, presence of neutropenia (neutrophil count, <500 cells/µl) at study-entry, underlying malignancy, site of catheter insertion, and number of catheter lumens. During follow-up, we recorded catheter dwell time, stay at the intensive care unit, treatment with total parenteral nutrition (TPN), and use of glycopeptides, which is the treatment for presumed/proven beta-lactamase resistant gram-positive microorganisms in our hospital. Safety data were registered for all patients, including all-cause mortality during the study episode until 30 days after catheter removal, and the discontinuation of study medication. Subjective parameters were recorded for a random sample of 25% of patients by means of a questionnaire.

In case of suspected CRBSI, definitions from current guidelines were followed.[8] In case of documented bacteremia or when a glycopeptide was started empirically, a culture of the inside of all catheter hubs was performed. The catheter insertion site was cultured in case of local inflammation or unexplained bacteremia. As our intervention would reasonably prevent only endoluminal CRBSI, effort was made to distinguish this modality. Strictly *endoluminal* CRBSI was defined as a positive central or peripheral blood culture with the same genotypic (for coagulase-negative staphylococci (CNS)) or phenotypic (for other microorganisms) strain cultured from the hub, for which directed antimicrobial therapy was started. For CNS or other skin-colonizers, ≥2 blood cultures had to be positive when no peripheral cultures were available.

In case of bacteremia, we calculated the differential time-to-positivity (DTTP), which denotes the difference in time-to-positivity of a peripheral blood culture minus the time-

to-positivity of a central blood culture. DTTP of ≥2 hours accurately predicts the catheter to be the source of the episode of bacteremia, which applies especially to patients with long-term catheters.[22,23] Therefore, CRBSI with DTTP of ≥2 hours in the absence of insertion site or tunnel infection was considered as a separate entity. When DTTP was not available, CRBSI was diagnosed in case of a positive peripheral or central blood culture with an identical microorganism detected on the catheter tip in the absence of any other infectious source. The latter two entities were defined as presumed endoluminal CRBSI, because strictly, these episodes cannot be diagnosed as endoluminal CRBSI with absolute certainty, although an endoluminal origin is more likely in the absence of signs of exoluminal infection. *Exoluminal* CRBSI was defined as bacteremia with negative hub cultures, but with the same strain cultured from blood and a clinically infected insertion site, combined with either DTTP of ≥2 hours or an identical microorganism detected on the tip.

Microbiological procedures

Regardless of suspicion of infection, catheter tips were processed by the semi-quantitative roll plate method.[24] After incubation for 72 hours, microorganisms were identified and quantified by standard microbiological methods. Catheter tip colonization was defined as a positive semi-quantitative tip culture of ≥15 colony forming units (cfu)/ml. Blood cultures were processed according to routine procedures (Bactec; BD).

For genetic typing of isolated CNS strains, we used arbitrarily-primed PCR, as described in detail elsewhere.[25,26] Strains were considered identical if all 3 primers showed corresponding DNA-fingerprints. When the strain was not available for genotyping, strains were considered phenotypically identical if antibiotic susceptibility patterns showed at maximum one disconcordant result.

Outcome

End points were reviewed by 2 blinded investigators (L.S. and B.J.R.). Patients with strictly endoluminal CRBSI and patients with presumed endoluminal CRBSI were considered as the primary outcome measure. The predefined secondary goals were to compare overall CRBSI (including exoluminal infection), overall bacteremia, incidence of positive hub and catheter tip cultures, all-cause mortality, and treatment with systemic antibiotics (glycopeptides versus other compounds) for both groups. Safety data were also assessed as a secondary outcome.

Statistics

Statistical analyses were performed using SPSS, version 15.0 (Chicago; Il). Tests were two-sided and a P value <.05 was considered statistically significant. Analyses were based on catheter episodes and performed on a modified intent-to-treat (ITT) population, consisting of all enrolled patients who received at least 1 dose of study-solution.

Follow-up was censored at the moment a primary endpoint was diagnosed, at catheter removal, or at death of patients.

Based on a comparable population, CRBSI was assumed to occur in ≥20%.[27] We assumed that the majority of CRBSI would be endoluminal CRBSI. Therefore, a sample size of 219 catheter episodes per group was calculated to detect a hypothesized 50%-reduction of endoluminal CRBSI with 80% power (α=.05). According to recommendations of the Centers for Disease Control and Prevention, we also determined CRBSI rates per 1000 CVC-days.[28] Kaplan-Meyer curves, to describe the rate of CRBSI for both groups as a function of time, were constructed and compared with log-rank tests.

The separate contribution of TPN, stay at the intensive care unit, underlying disease, neutropenia at time of catheter insertion, catheter insertion site, and number of catheter lumens was assessed in a Cox regression model. For secondary endpoints, differences between groups were analyzed with chi-square tests or Fisher's exact tests in case of dichotomous variables; differences in means were compared with student's t-tests, as appropriate.

Results

A total of 379 patients were enrolled, accounting for 453 catheter episodes. No study solution was administered in 5 catheter episodes, so the modified ITT-analysis was based on data obtained from 448 episodes (376 patients). Ethanol locks were administered in 226 catheter episodes. Characteristics of the 2 groups are summarized in table 1.

Prophylactic effect of 70%-ethanol lock on CRBSI

The differences between the rates of endoluminal CRBSI in both groups were not statistically significant (table 2). For ethanol locks, a total of 14,262 catheter days and 10 episodes of endoluminal CRBSI were recorded, accounting for a rate of 0.70 CRBSIs per 1000 CVC-days (95% confidence interval, 0.4-1.3). For placebo, 16 endoluminal CRBSIs during 13,483 catheter days were observed, with a rate of 1.19 CRBSIs per 1000 CVC-days (95% confidence interval, 0.7-1.9). The calculated incidence rate ratio was 0.59 (95% confidence interval, 0.27-1.30), which implies a non-significant reduction of 41% for patients treated with ethanol locks (P=.19). In figure 1, Kaplan-Meyer curves are presented to describe the rates of CRBSI as a function of catheter dwell time. No significant difference was observed when these curves were compared with log-rank tests (P=.22). In patients who classified for endoluminal CRBSI according to the strictest definition (positive hub culture with identical bacterial strain in blood), a 3.6-fold reduction was observed for patients allocated to ethanol locks (2 of 226 versus 7 of 222; P=.103).

Table 1. Patient characteristics.

	Ethanol (n=226)	Placebo (n=222)
Baseline		
Age, mean years (range)	51.7 (18-75)	49.8 (18-74)
Male sex	130 (57.5)	125 (56.3)
Neutropenia[a] at insertion	44 (19.5)	47 (21.2)
Underlying malignancy		
AML-MDS or ALL	140 (61.9)	119 (53.6)
Other	86 (38.1)	103 (46.4)
Type of central venous catheter		
Double-lumen	83 (36.7)	99 (44.6)
Triple-lumen	139 (61.5)	122 (55.0)
Missing data	4 (1.8)	1 (0.4)
Insertion place		
Internal jugular vene	214 (94.7)	218 (98.2)
Subclavian vene	5 (2.2)	0 (0.0)
Femoral vene	1 (0.4)	1 (0.4)
Missing data	6 (2.7)	1 (0.4)
Follow-up		
Catheter dwell time, mean days (range)	63.1 (2-486)	60.7 (4-308)
Total parenteral nutrition	117 (51.8)	91 (41)
Stay at intensive care unit	18 (8.0)	13 (5.9)

Note. Data represent numbers (%) of patients unless indicated otherwise. AML-MDS, acute myeloid leukemia-myelodysplastic syndrome; ALL, acute lymphoblastic leukemia.
[a]Neutrophil count, <500 cells/µl.

Neither treatment with TPN nor stay at the intensive care unit, underlying disease, neutropenia at time of catheter placement, catheter insertion site, or number of catheter lumens contributed individually to the development of CRBSI (data not shown).

Table 2. Overview of endpoints and other parameters.

	Ethanol (n=226)	Placebo (n=222)	P
Strictly endoluminal CRBSI	2	7	.10
Presumed endoluminal CRBSI	8	9	.81
Combined primary endpoint	10	16	.23
Primary bacteremia	91	91	.95
Positive culture of catheter hub[a]	8	11	.67
Positive culture of catheter tip[b]	49	57	.52
Exoluminal CRBSI	11	8	.64

Note. Data represent numbers of events. CRBSI, catheter-related bloodstream infection.
[a]Positive catheter hub cultures, performed during episodes of bacteremia (n=73 for ethanol; n=74 for placebo).
[b]Positive results of overall catheter tip culture (n=171 for ethanol; n=176 for placebo).

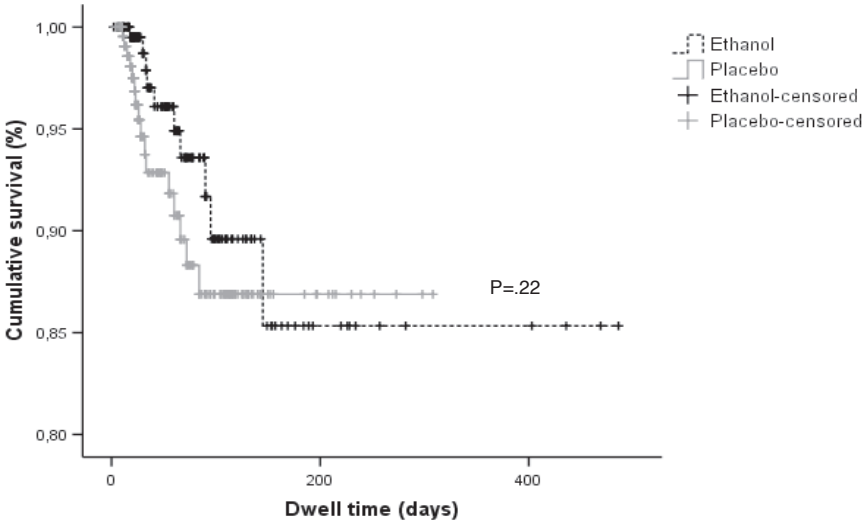

Figure 1. Kaplan-Meyer survival curves for comparison of the rate of catheter-related bloodstream infection for patients treated with an ethanol lock (n=226) or placebo (n=222) as a function of catheter dwell time.

Secondary goals

Results are presented in table 1 and 2. The mean catheter dwell time was 63.1 days (range, 2-486 days) for ethanol locks versus 60.7 days (range, 4-308 days) for placebo (P=.71). The mean duration of the use of a glycopeptides (6.0 versus 5.0 days) did not differ between patients randomized to ethanol locks or placebo (P=.62). Also, the duration of treatment with other classes of systemic antibiotics did not differ between ethanol locks (mean duration, 17.4 days), or placebo (mean duration, 16.7days). Overall CRBSI was recorded in 21 of 226 patients allocated to ethanol locks versus 24 of 222 patients treated with placebo (P=.71). For overall bacteremia, these results were 91 of 226 versus 91 of 222 patients, respectively (P=.95).

Tip cultures were performed on 347 catheters. Rates of detection of microbial growth were 49 of 171 in patients treated with ethanol locks and 57 of 176 in patients allocated to placebo (P=.52). Of all catheter episodes in which bacteremia was documented (n=182), CNS was detected as the causative pathogen in 106 episodes (58%), which was equally distributed between both groups, as were other causing microbes (table 3). Hub cultures were performed in 147 patients, and were positive in 8 patients in the ethanol arm versus 11 patients in the placebo arm (P=.67). However, different strains were obtained from hubs as compared with blood cultures in 4 patients, (n=2 for ethanol and placebo). Therefore, not all patients with positive hub cultures qualified for endoluminal CRBSI.

Table 3. Overview of cultured microbes in case of bacteremia (182 episodes).

	Ethanol (n=91)	Placebo (n=91)
CNS[a]	49	57
Other skin colonizers	2	2
Staphylococcus aureus	2	3
Other gram-positive cocci	12	10
Gram-negatives	4	5
Polymicrobial	20	13
Yeasts	2	1

Note. Data represent numbers of episodes of bacteremia. CNS, coagulase-negative staphylococci.
[a]Of all 106 episodes with CNS-bacteremia for which glycopeptide-therapy was started, 32 were due to CRBSI (including endoluminal and exoluminal infection). Of the remaining 74 episodes, tentative sources were mucositis (n=21), cytarabin skin toxicity (n=8), contaminated blood cultures (n=8), red catheter insertion site without other criteria for exoluminal CRBSI (n=6), unknown (n=29), and other causes (n=2).

Safety and tolerability aspects

Data are presented in table 4. All-cause mortality in patients allocated to ethanol locks was 7 of 226, compared with 5 of 222 patients randomized to placebo (P=.77). None of the involved deaths were diagnosed with CRBSI during the catheter episode.

Table 4. Tolerability and safety of study compound.

	Ethanol (n=226)	Placebo (n=222)	P
Total cohort			
All-cause mortality	7	5	.77
Thrombosis of insertion blood vessel	9	12	.62
Discontinuation of study compound			
Modified lock frequency	10	0	.002*
Complete cessation	11	1	.006*
Other events[a]	2	0	.50
Questionnaire sample[b]	(n=88)	(n=93)	
Subjective parameters			
Facial flushing	39	17	<.001*
Nausea/vomiting	20	17	.58
Altered taste	31	19	.04*
Sensations of dizziness/drowsiness	41	10	<.001*

Note. Data represent numbers of events; *denotes statistical significance.
[a]One patient had syncope right after flushing the first lock solution into the circulation, 1 device had to be removed because of a rupture of a catheter lumen which occurred during sleep.
[b]The predefined analysis of subjective adverse effects was performed on a random sample of the total cohort.

No differences were observed in the incidence of thrombosis. In patients allocated to ethanol locks, 1 device had to be removed because of a rupture of 1 of the 3 catheter lumens, which occurred while the patient was asleep. No life-threatening adverse events were observed. One ethanol-treated patient had syncope shortly after flushing through the first lock solution. During subsequent ethanol lock procedures, no further adverse effects occurred in this particular patient. Significantly more patients receiving ethanol locks discontinued lock therapy (P=.006) or continued with a frequency-adjusted regimen (P=.002), as compared to placebo. This was due to subjective feelings of discomfort, including facial redness or flushing, feelings of drowsiness or an alcohol taste after flushing the lock solution through. No differences in levels of hepatic enzymes (aspartate-aminotransferase, g-glutamyl transpeptidase) and mean corpuscular volume of red blood cells were observed after 2 weeks of lock therapy when compared to baseline values (P>.5 for all parameters; data not shown).

Discussion

The present randomized clinical trial on the use of a preventive ethanol lock showed a non-significant 41%-reduction of endoluminal CRBSI in patients allocated to ethanol locks for occurrence of CRBSI as expressed per 1000 CVC-days. Also, the 3.6-fold reduction as observed in ethanol lock patients who classified for endoluminal CRBSI according to the strictest definition was not significant. No differences were observed for catheter dwell time, use of glycopeptides and other systemic antibiotics, and rates of overall CRBSI or bacteremia between groups.

In patients treated with ethanol locks, 1 device had to be removed due to loss of integrity of the CVC; another person experienced an episode of syncope after the first lock procedure but not after subsequent procedures. No other serious adverse events were observed, which is in agreement with other reported data.[20] More patients treated with ethanol locks discontinued their prophylactic treatment. All reported adverse effects were non-severe but reasonably ethanol related. In future studies, this may partially be circumvented by removing the lock solution instead of flushing it through, as has safely been done in other recent studies.[21]

We took efforts to perform a double-blind, randomized trial. However, due to the specific ethanol odour that could be sensed after opening the ampoules by the nursing team, blinding was not 100% in daily practice. Nevertheless, the principal investigators were not directly involved in patient management and were therefore completely blinded at all time. Furthermore, the primary endpoint has no subjective element in its definition, which may reasonably minimize potential bias.

Currently, several promising observational in vivo data on the *treatment* of CRBSI with ethanol locks have been reported.[15-17] Overall tolerance of ethanol was good in these studies and no significant adverse effects were observed. Furthermore, several case-series on the use of *preventive* ethanol locks have been published. In a recent case-series, Mouw and colleagues described 10 TPN-dependent pediatric patients with tunnelled catheters, who were treated with a 70%-ethanol lock solution between TPN infusions.[19] Infection rates in 5 children of whom data were available from the period before initiation of lock therapy declined from 11.2 to 2.1 CRBSIs per 1000 CVC-days. In a recent small randomized trial, Sanders and colleagues observed a reduced incidence of CRBSI with a 70%-ethanol lock in hematology patients with tunnelled CVCs.[21] CRBSI occurred in 3 versus 11 patients in the ethanol and control groups, respectively (odds-ratio, 0.18; 95% confidence interval, 0.05-0.65). Catheter survival was longer in the ethanol group (P=.003). Several differences with our study should be taken into account. First, Sanders et al. used less stringent CRBSI definitions. With this respect, it is surprising that the large majority of CRBSIs was caused by gram-negative microorganisms instead of staphylococci. One wonders whether these episodes of bacteremia were the consequence of translocation from the gut rather than CRBSI. The lack of stringent definitions may also partly explain the high incidence of CRBSI (31 per 1000 CVC-days) in the control group, which is around 16 times lower in our present study (1.19 per 1000 CVC-days) and another landmark study.[28] Interestingly, the preliminary data of a randomized trial performed by Crnich and colleagues, including 359 long-term tunnelled or implanted CVCs, showed no benefit of the use of a 50%-ethanol lock for CRBSI-prevention in hospitalized patients.[29]

Ethanol acts bactericidal and fungicidal against a broad range of bacteria and even yeasts without concerns of resistance development.[18] In vitro, it has been demonstrated that a 15%-ethanol concentration was able to kill most planktonic microorganisms[30] For microorganisms in established biofilms, which is the case in CVCs, concentrations of 40% to 70% were required to achieve a bactericidal effect because penetration into a biofilm is harder to establish.[31] As a concern, it has been reported that a 100%-ethanol lock solution was associated with catheter occlusion.[32] Another report showed that infusion of polyurethane catheters with 70%-ethanol resulted in qualitative softening of the catheters.[33] More recently, however, no changes were observed on the biomechanical properties of polyurethane catheters, which were submerged in an ethanol solution for 9 weeks.[34] An overview of all recent studies, discussing the most relevant aspects of the ethanol lock technique was published recently.[35]

Several factors may explain the lack of efficacy as observed in our study. First, for practical reasons we used a lock time of 15 minutes daily per catheter lumen. This was

decided because a longer dwell time would have interfered too much with patient care. A recent in vitro study showed that a significant 3-log reduction in the number of biofilm-associated gram-positive cocci occurred already after 20 minutes exposure to a 60%-ethanol lock solution. A dwell time of 30 minutes was required for complete eradication.[36] However, another in vitro study showed recently that an exposure time of 1 minute to a 70%-ethanol solution was sufficient for the sterilization of a bacterial biofilm.[37] Second, a lock-based intervention will reasonably prevent only *endoluminal* CRBSI. By employing strict definitions we tried to differentiate endoluminal CRBSI from other entities. However, the true sensitivity of hub cultures to detect endoluminal infection is unknown. Finally, bacteremia with CNS is not always CVC-related, but may result from translocation from the bowel in patients with severe mucositis.[38,39] This could explain why despite the high overall incidence of CNS bacteremia, which occurred in 106 of all 182 episodes of bacteremia, no reduction of CNS bacteremia was seen due to the use of ethanol locks. The use of antimicrobial prophylaxis resulting in selective eradication of intestinal gram-negative but not gram-positive microorganisms may be the underlying cause. To test this hypothesis, we did genotypic identification of CNS in a random sample of 15 patients with documented bacteremia who were found to have concomitant CNS in rectal and/or vaginal mucosa or mouth swabs. Identical CNS strains in blood and mucosa were identified in 6 of 15 patients (40%).

Although the relevance of mucositis-associated bacteremia is not fully elucidated yet, it may be hypothesized that an intervention with an endoluminal CVC lock will not result in a reduction of *overall* bacteremia in patients who are treated with high-dose chemotherapy, and nearly inevitably suffer from severe mucositis. Also, the observed rate of exoluminal compared to endoluminal CRBSI in our study was higher than expected. Both these aspects may explain why our initial hypothesis that the rate of CNS bacteremia as observed in other studies reflects mainly *endoluminal* CRBSI may have been inaccurate in retrospect. In this view, the observed 3.6-fold reduction of strictly endoluminal CRBSI in patients allocated to ethanol locks is reassuring, as is the 41%-reduction of endoluminal CRBSI as expressed per 1000 CVC-days, because the lack of statistical significance may reflect a lack of power more than a lack of effectiveness.

However, the overall incidence of endoluminal CRBSI in our patients was low. One wonders whether the clinical benefits of this intervention, even in case of a significant reduction of endoluminal CRBSI, in this specific patient population would outweigh the extra amount of effort, costs and patient discomfort. Additional studies should therefore be performed in populations with higher incidence of (endoluminal) CRBSI, e.g., patients receiving long-term treatment with TPN.

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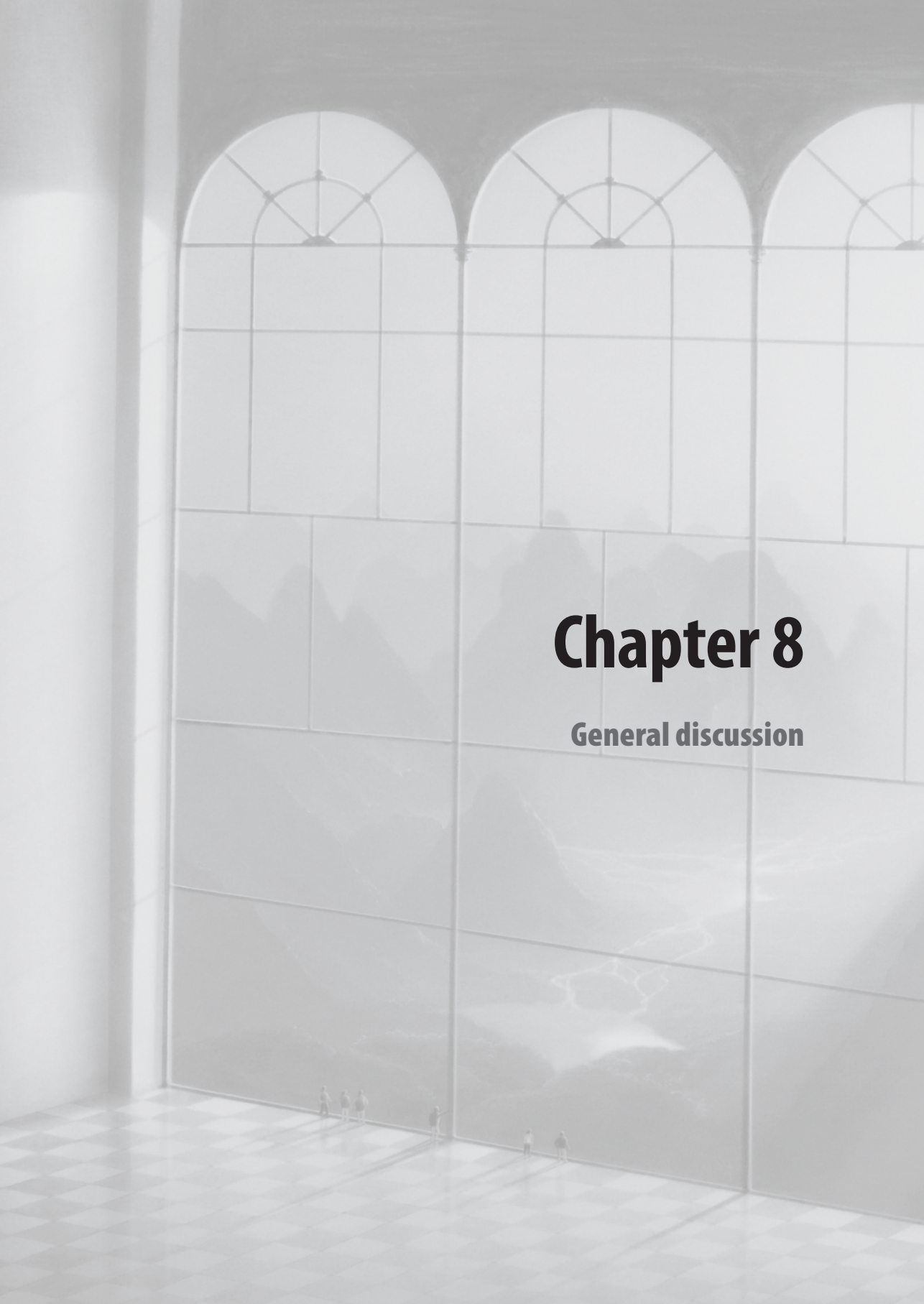

Chapter 8

General discussion

Thanks to a lot of pioneering work during the last few decades, many treatment strategies have been developed for patients with hematological malignancies.[1,2] However, despite major improvements of the prognosis and life expectancy of these patients, the intensive courses of chemotherapy also created various new hurdles to deal with.[3-5] The disturbed immunologic competence in case of severe neutropenia or suppression of T-cell function for the prevention or treatment of complications of hematopoietic stem cell transplantation, combined with the disruption of the host's mechanical defence barriers puts patients at risk for severe infectious complications of various etiology. Thus, further survival improvement substantially relies on the management of these infections, which hamper anti-leukemic treatment. In this thesis, the results of several investigations to the prevention of infectious complications in patients who are treated with intensive chemotherapy for hematological malignancies were discribed.

Invasive pulmonary aspergillosis: scientific background and study rationale

In **Part I**, we focussed on several aspects on the prevention of invasive pulmonary aspergillosis (IPA). The incidence of IPA has increased since the introduction of aggressive cytotoxic chemotherapy and the use of high-dose corticosteroids as a treatment modality.[6-8] There are several populations with well-known risk factors for the development of IPA. These include patients with prolonged severe neutropenia due to either leukemia or treatment with high-dose chemotherapy, and patients with T-cell and neutrophil dysfunction caused by immunosuppressive therapy (especially corticosteroids), used during and/or after hematopoietic stem cell or solid organ transplantation. The role of the human immune system in the defence against IPA has been discussed in more detail in Chapter 1. However, an increased incidence of IPA is currently observed in less well-established risk populations. For example, the use of alemtuzumab has been associated with an increased amount of infectious complications, including a 4% to 7% incidence of invasive aspergillosis.[9,10] Alemtuzumab is a monoclonal antibody targeted against CD-52, which is a glycoprotein expressed at the surface of various hematological cells, including T- and B-lymfocytes, and is used in the treatment of chronic lymphocytic leukemia or T-cell lymphoma. In another study among 1850 Belgian patients at the intensive care unit, an incidence rate of invasive aspergillosis of 6.9% (127 patients) was observed.[11] Of these 127 patients, 89 patients had no hematological malignancy. Among these cases, 35 had chronic obstructive pulmonary disease, 6 suffered from liver cirrhosis, and 22 were classified as miscellaneous. None of these were patients with the classical risk factors mentioned above. The remaining 26 patients had well-known risk factors, like a solid organ transplant or autoimmune diseases, requiring immunosuppressive therapy. The overall mortality in these 89 patients was 80%.

Important improvements in the diagnosis of IPA have been made, e.g., high-resolution CT of the lungs, polymerase chain reaction (PCR) for the detection of fungal DNA, and an enzyme immunoassay (EIA) to detect galactomannan levels, which is a component of the fungal cell wall, in bronchoalveolar lavage (BAL) fluid or serum specimens.[12-21] However, despite these techniques and regardless the recent development of effective treatment modalities, considerable incidence rates with high case-fatality rates are still reported among hematology patients with prolonged neutropenia and/or hematopoietic stem cell transplantation.[7,8,22-25] Therefore, there is still an urgent need for efficacious prevention strategies with more favorable safety profiles.[26]

Recent data on the prophylactic use of orally administered posaconazole, a new broad-spectrum azole, were promising.[27] In a randomized, multi-center study, patients treated with chemotherapy for acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) received oral prophylaxis during each cycle of chemotherapy until they recovered from neutropenia and were in complete remission, until occurrence of an invasive fungal disease, or for up to 12 weeks, whichever came first. A total of 304 patients were assigned to receive prophylaxis with posaconazole 200 mg thrice daily, 240 patients were randomized to prophylactic treatment with fluconazole 400 mg once daily and 58 patients received itraconazole 200 mg twice daily. Proven or probable invasive fungal infections were observed in 7 patients (2%) in the posaconazole arm versus 25 patients (8%) in the subset treated with fluconazole or itraconazole (95% confidence interval for absolute reduction in the posaconazole group, –9.7% to –2.5%; P<.001). Significantly fewer patients in the posaconazole arm had invasive aspergillosis (2 versus 20 patients; P<.001) and survival was longer (P=.04). Serious adverse events that were possibly or probably related to administration of the study drug were more reported in the posaconazole group as compared to patients allocated to itraconazole or fluconazole (P=.01).

However, decreased susceptibility of *Aspergillus fumigatus*, the most commonly encountered species, for azoles is increasingly reported.[28] The prevalence of itraconazole resistance was investigated in 1912 clinical isolates of *Aspergillus fumigatus*, collected between 1994 and 2007 and obtained from 1219 patients. Itraconazole-resistant isolates were observed after 1999 in a total of 32 patients, with an annual incidence varying from 1.7% to 6.0%. Resistant strains also showed elevated minimal inhibitory concentrations for voriconazole and posaconazole. Itraconazole-sensitive strains were not more likely to be responsible for invasive aspergillosis than resistant isolates. It is therefore unclear whether extensive prophylactic use of azoles will remain a viable option in the future.

Another drawback of systemic azole therapy is the potential for sometimes life-threatening drug-drug interactions, as many co-administered drugs are metabolized through the cytochrome P-450 metabolic pathway. A preventive strategy that allows the

avoidance of systemic drug exposure would therefore be welcome. A drug that retains activity against azole-resistant *Aspergillus* strains would be an ideal candidate.[27,29-31] Intravenously, the conventional formulation of the fungicidal polyene amphotericin B is known to be effective in the treatment of IPA, although its use is hampered by many adverse effects. Adverse effects are less frequent with lipid formulations of amphotericin B, especially liposomal amphotericin B.

Because the inhalation of airborne *Aspergillus* conidia in the lungs is the first step in the pathogenesis of IPA, an inhaled formulation of an antifungal agent will attack the infection at the level where it originates. Concurrently, inhalation of the drug will minimize systemic adverse effects. Encouraging results on the prevention of IPA with inhalation therapy in animal models have previously been described.[32-36] Nevertheless, results of a multi-center trial on the use of aerosolized conventional amphotericin B did not demonstrate a positive effect on prevention of IPA. However, overall event rates in this study were low, precluding definite conclusions.[37]

Intravenously, liposomal amphotericin B has fewer adverse effects than conventional amphotericin B. This is also reported for aerosolized liposomal formulations as compared to conventional formulas, although this advantage was not observed in all studies.[38,39] The better tolerance of liposomal amphotericin B could be explained by the fact that the liposomal carrier exhibits a surfactant-like function. In contrast, the conventional formula is based on a deoxycholate salt, which acts as a detergent. In vitro, this was shown to have a detrimental effect on the function of pulmonary surfactant by lowering the surface tension.[34,36] Apart from tolerability aspects, experimental data on the prevention of IPA derived from animal models have suggested higher activity for aerosolized lipid compared to conventional formulations of amphotericin B, possibly due to higher drug retention and better lung deposition.[32,33]

Efficacy and safety of liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis

Therefore, liposomal amphotericin B was selected for use in our clinical study, which was the first randomized, human trial using liposomal amphotericin B for this purpose (Chapter 2). In this placebo-controlled study on the prevention of IPA in hematology patients, aerosolized liposomal amphotericin B was administered for two days a week during course-related prolonged neutropenia. The intent-to-treat analysis of the primary endpoint in our study showed a statistically significant and promising reduction of probable and proven cases of IPA, which was defined according to the diagnostic criteria of the European Organisation for Research and the Treatment of Cancer-Mycoses

Study Group (EORTC-MSG).[40,41] We observed 18 IPA cases among 132 patients treated with placebo (14%) versus 6 among 139 patients (4%) allocated to aerosolized liposomal amphotericin B (P=.005). Results for on-treatment analyses and secondary endpoints, using modified definitions were equally encouraging.

None of the 30 allogeneic stem cell transplantation recipients developed IPA. Although bone marrow transplantation recipients are well-known risk patients and IPA may be diagnosed in the first few weeks after transplantation (especially in case of graft failure or delayed engraftment), the majority of IPA cases is diagnosed in patients who suffer from graft-versus-host disease (GvHD), necessitating treatment with high-dose corticosteroids.[7,18,42,43] In our current study, inhalation with liposomal amphotericin B was continued until recovery from neutropenia. Therefore, conclusions on the efficacy of aerosolized liposomal amphotericin B for the prevention of GvHD-related IPA cannot be drawn.

No systemic toxicity was observed when baseline versus post-nebulization serum levels of renal function and hepatic enzymes were compared (Chapter 3). However, one would expect no differences, as aerosolized liposomal amphotericin B is not supposed to reach systemic drug levels, which has been demonstrated in other studies.[39,44-46] Overall tolerability was reasonably good. Only coughing was reported more significantly among patients treated with liposomal amphotericin B, without other major treatment-limiting adverse effects. No severe bronchospasm occurred, and no pre-inhalation use of salbutamol was required. Although other tolerability studies differ in design, study population, sample size, and duration of amphotericin B inhalation, and sometimes used other lipid formulations, our results are generally in agreement with data reported from these studies.[38,39,46,47]

We also studied the effects of aerosolized liposomal amphotericin B on the forced expiratory volume in 1 second (FEV_1) and the forced vital capacity (FVC), two parameters of the lung function. Between groups, differences were neither observed in the proportion of patients with pulmonary function decline nor in the magnitude of decline. As a remark, a rate of 8 of 31 patients (21%) allocated to placebo had a significant decline of post-nebulization lung function compared with pre-nebulization values at some time during their entire inhalation episode, compared with 12 of 38 patients (32%) in patients treated with liposomal amphotericin B (P=.20). A post-nebulization decline in lung function was not related to a decline after other inhalations in the same patient, which would be expected in case of a causative relationship with the study compound. This probably illustrates the difficulty and maybe even unreliability of the use of an otherwise well-validated test when used during non-optimal test circumstances. We think that the use of relative instead of absolute values as well as the comparison between

pre-nebulization and post-nebulization values has probably circumvented some of these limitations.

The overall incidence rate of IPA among hematology patients as observed in our hospital is in the upper range as reported in the literature.[7] Incidence rates are known to vary considerably among different centers and even within individual centers.[7,25] In the placebo group of our quite heterogeneous prophylaxis study, an incidence rate of 14% was observed. However, in the study on the outcome and costs of IPA patients, including solely patients with AML or MDS, an incidence of probable/proven IPA of 18% was observed. The incidence was even 30% when possible cases were included as well (80 of 269 patients), which is in agreement with the rate among the subgroup of AML-MDS patients in our prophylaxis study.

The hospital construction and demolition works in 1 of the 2 locations of our hospital cannot be blamed, as the incidence did not differ between the 2 buildings. However, other factors may serve as a partial explanation for the high incidence. In our study, patients were followed during their entire anti-leukemic treatment period in our study, whereas patient follow-up in other studies ended at remission of leukemia.[27] Second, the higher incidence probably reflects the yield of our scrutinized diagnostic protocol for patients with unexplained fever. This consisted of routinely performing a BAL in case of documented radiological abnormalities on high-resolution CT of the lungs. A high-resolution CT was performed if fever during neutropenia persisted for 5 days despite treatment with empirically started broad-spectrum antibiotic therapy. Using the updated EORTC-MSG definitions, a combination of 3 criteria is required to diagnose probable IPA.[41] Physicians are often reluctant to perform biopsies of suspected radiological abnormalities in patients with chemotherapy-induced profound thrombopenia and neutropenia, as such procedures can be complicated by bleeding and infection. However, the lack of pathological evidence in the majority of patients with suspected IPA hampers patients to be diagnosed with proven IPA, as this requires a positive culture or the demonstration of invasive growth of *Aspergillus* hyphae in a biopsy obtained from a radiologically abnormal site. Therefore, diagnosing IPA mostly relies on the demonstration of probable IPA according to EORTC-MSG definitions. According to these definitions, a host factor together with clinical or radiological signs, and a microbiological factor have to be present. The host factor is often the prolonged use of corticosteroids and/or neutropenia, and the clinical criterion is usually the presence of suggestive radiological abnormalities like a well-described nodule with or without a halo sign, an air-crescent sign, or a cavity within an area of consolidation. Finally, the microbiological criterion can be either a positive culture of *Aspergillus,* or the detection of galactomannan, an *Aspergillus* antigen derived from the fungal cell wall, which can be measured in serum

samples or BAL fluid specimens. Until now, PCR is not validated or standardized enough to be incorporated in current definitions.[41]

Several studies have demonstrated the additional yield of using galactomannan levels detected in BAL fluid instead of serum or plasma samples.[16-19] The sensitivity of galactomannan measurement in BAL for the diagnosis of IPA has been demonstrated to be higher than serum galactomannan.[17,18,21,48] In our prophylaxis trial, the microbiological component of the diagnosis was based on BAL galactomannan in 21 out of 24 IPA cases. Four patients had both positive serum and BAL values and in only 3 patients, the mycological proof was a positive serum galactomannan only. The systematic use of bronchoscopy in patients with pulmonary abnormalities on high-resolution CT permits galactomannan measurement in BAL fluid samples as the microbiological criterion. Without this, many cases would have remained possible IPA rather than probable because of the lower sensitivity of serum galactomannan. In contrast to our diagnostic approach, many centers administer empirical antifungal therapy to AML patients provided that the episode of neutropenic fever has not disappeared after several days of treatment with broad-spectrum antibiotic therapy. Following this approach, many cases of IPA may remain undiagnosed, especially in a hospital setting in which high-resolution CT and autopsies are not performed routinely. To summarize, there are many pitfalls when one compares incidence data of IPA between different hematology centers.

Our mortality data compare favorably with literature data (Chapter 4). Although again different in design and study population, other landmark studies reported a 12-week overall mortality of 29% versus 42% (voriconazole intravenously (iv) versus conventional amphotericin B iv), 34% (liposomal amphotericin B iv) or 46% (caspofungin iv).[23,24,49] In our study, an all-cause mortality rate of 16 of 73 patients (22%) after 12 weeks of antifungal therapy was registered for patients with possible and probable/proven IPA, with an estimated attributable mortality for IPA of 10%. Our diagnostic-driven approach, which results in early diagnosis and treatment in most cases of IPA, may account for this, as an early diagnosis has been demonstrated to influence the prognosis favorably.[50] Of note, in contrast to current recommendations, voriconazole treatment was preferentially started orally instead of iv, which we considered as a safe option because of the excellent bioavailability of the oral formulation.

Even when the efficacy of liposomal amphotericin B inhalation for the prevention of IPA is established, clinicians will have to consider for which patient populations inhalation should be recommended. Given that hospital resources are not infinite, it is important to evaluate the cost-effectiveness of any IPA-preventive approach. In our study, the corrected additional hospital-based management costs in AML-MDS patients with IPA were €8360 and €15,280, respectively, for patients with possible IPA versus patients

with probable/proven IPA, compared with patients that were free of IPA (Chapter 4). Of note, costs were not influenced by the severity of the underlying leukemia or the duration of the anti-leukemic treatment, but were specifically related to the presence of IPA. The same observation has been made in a pediatric study.[51] Second, these are just economic considerations from a hospital perspective, which do not take into account other important aspects from a societal point of view.

These extra management costs pave the road for cost-effective implementation of IPA prophylaxis in general, and prophylactic inhalation of liposomal amphotericin B in particular, in high-risk patients. In a cautious estimation, we calculated that implementing prophylaxis with aerosolized liposomal amphotericin B would be even money saving. To clarify this, analyses like Markov-modelling should be performed, but a formal cost-effectiveness study would provide a more definitive answer.

Possible directions for further research

One should notice that prophylactic inhalations were administered in our study for a maximum of 6 weeks. Therefore, data on the long-term safety cannot be derived from our observations. However, for most of the currently imaginable indications of aerosolized liposomal amphotericin B, one could probably suffice with short-term administration, unless it would be considered for preventive use in patients with a chronic severe immunodeficiency or patients with chronic GvHD after allogeneic hematopoietic stem cell transplantation. Other patients at relatively high risk for IPA are patients with chronic granulomatous disease and patients with a lung transplant, especially in case of transplant rejection, necessitating the long-term use of higher doses of immunosuppressive medication. However, because GvHD can also involve the lungs, and allogeneic stem cell transplantation can be complicated by other pulmonary complications, e.g., bronchiolitis obliterans organizing pneumonia (BOOP), it is clear that the lung safety of aerosolized liposomal amphotericin B in this population should be studied in more detail before it can be implemented as a universally accepted intervention.

Practical hurdles have to be taken before the long-term preventive use of inhalation of liposomal amphotericin B can be implemented in common practice. Reasonably, tolerability may be further improved if the inhalation frequency could be reduced without a reduction of efficacy. In an animal model, a beneficial effect on survival could still be observed 6 weeks after inhalation of one single dose of liposomal amphotericin B.[52] In our opinion, the most straightforward way to determine the minimal required inhalation frequency needed for protection against IPA will be the measurement of lung tissue levels of amphotericin B at different time points after inhalation. This could be performed in

patients being planned for (partial) lung resection, e.g., for the treatment of lung cancer, who agree to inhale liposomal amphotericin B preoperatively for study purposes.

Also, progression must be made in the development of cheaper, easy-to-handle nebulization devices, which are also suitable for out-of-hospital use, analogously to other diseases, like cystic fibrosis. Promising data were recently observed for amphotericin B inhalation powder (ABIP), a novel highly respirable dry powder formulation. ABIP can be administered with a proprietary handheld dry powder inhalation device and is formulated to have an aerodynamic particle size similar to fungal spores. This results in targeted drug delivery to the same airways and alveolar surface on which fungal spores deposit and germinate. Plasma total amphotericin B concentrations in 35 healthy volunteers (dose groups of 5, 10 and 25 mg) exhibited a 1-2 hour post-dose delay in appearance, and peaked around 8 hour, but were well below those normally associated with systemic amphotericin B toxicity, as seen with intravenous amphotericin B use. Overall tolerance was good, although non-severe cough, dizziness, headache and dysgeusia were reported. As in our study, spirometric changes were mild, transient and not clinically significant.[53] Studies of ABIP in laboratory animals showed that the half-life in lung parenchyma is around 20 days.

ABIP was administered as a single-dose inhalation 1 day or 12 days before inoculation with *Aspergillus fumigatus* spores in a neutropenic rabbit model.[54,55] In this model, 80% of unprotected rabbits were found dead or moribund by day 14, while only 30% of the ABIP-treated rabbits succumbed, a rate equivalent to the controls (40% mortality). Kaplan-Meier analyses of these results showed that this effect was highly significant (P=.0089). No survival differences were observed between rats which inhaled 12 days versus 1 day before inoculation with *Aspergillus* spores, calling for possible considerations of infrequent dosing intervals. Unfortunately, at this time it is unclear whether further clinical development of this inhalation powder will be continued.

One must consider that prophylactic inhalation of aerosolized liposomal amphotericin B has to be administered on top of the entire anti-leukemic treatment to all patients, because until now it remains impossible to accurately predict which high-risk patient will eventually develop IPA. One aspect that may be taken into account is that in our study 75% of IPA-positive patients developed the disease during the first course of chemotherapy. This may lead to the adaptation that patients only receive prophylaxis during their first course. Further, more sophisticated modifications may be possible in the nearby future. Recently, several studies showed promising data on genetic polymorphisms that seem to be associated with increased susceptibility to IPA.[56-58] Haplotype analysis of interleukine-1 revealed that a VNTR2/-889C/-511T haplotype was strongly associated with susceptibility to develop IPA infection. It also showed an association between a VNTR2/-889C/-511C haplotype and resistance to IPA infection. Furthermore,

patients with IL1Ra VNTR2/2 and IL1β-511T/T genotypes had a higher positive serum galactomannan percentage compared with patients who had other genotypes.[58] Also, polymorphisms of chemokine-ligand 10 with C-X-C motif (CXCL10) were significantly associated with development of IPA in another study.[57] CXCL10 plays a role as an inflammatory mediator, induced by interferon-γ, and stimulates the directional migration of Th_1 cells as well as increasing T-cell adhesion to endothelium. Serum samples from patients with probable and proven IPA showed increased levels of CXCL10 compared with immunosuppressed patients without IPA. However, immature dendritic cells exposed to *Aspergillus* spores showed markedly elevated levels of CXCL10 if carrying the wild haplotype. Finally, 2 donor single-nucleotide polymorphisms (SNPs) in the Toll-like receptor 4 (TLR4) gene were associated with development of IPA in recipients of allogeneic hematopoietic transplantation in another study.[56] Together, these data suggest that, in time, it may be possible to define IPA-susceptible patients based on the presence of a panel of polymorphisms. This possibly implies that only a subset of patients undergoing high-dose chemotherapy of hematopoietic stem cell transplantation will need to be given antifungal prophylaxis, which would further increase cost-effectiveness.

In summary, future progress in the fight against *Aspergillus* mould infections can be anticipated with an integrated approach of *preventing* disease in high-risk patients, *diagnosing* infections in early stages and improving *treatment* outcome with currently available agents in case of documented infection. If confirmed in future studies, we consider the prophylactic administration of short-term aerosolized liposomal amphotericin B to be an attractive and safe *preventive* approach, as an alternative to systemic prophylaxis with azoles or even echinocandins. This strategy has been implemented in our hospital since the end of 2008 as the standard of care for all AML-MDS patients during induction and consolidation chemotherapy, as well as for patients with acute lymphoid leukemia during induction courses, as the highest incidence rate of IPA was observed among these particular subset of patients in our prophylaxis trial. Results will be evaluated by means of a formal cost-effectiveness analysis and are awaited in 2011. In the mean time, our current *diagnostic* approach in case of unexplained fever in neutropenic hematology patients seems to be satisfactory, emphasizing the merits of early high-resolution CT of the lungs and performing a BAL in case of pulmonary abnormalities. Further, new diagnostic strategies that could help to diagnose IPA before it becomes clinically obvious, possibly including the use of serial-PCR or β-1,3-D-glucan testing on blood, are awaited. Finally, new and more effective *treatment* modalities to improve the outcome, including research on combination therapy are eagerly awaited in view of the still considerable mortality in high-risk patient categories. Experimental *in vitro* and *in vivo* research in our hospital is currently performed with promising new agents, like chitin-synthase inhibitors, aimed at decreased production of chitin, a

glycopolymer, which is an important component of the fungal cell wall. These agents could be combined with echinocandins which also attack the fungal cell wall by inhibition of 1,3-β-glucan synthase, a key-enzyme involved in the synthesis of β-glucan.[59-61] Also, a worldwide industry-driven clinical multi-center study on the combination of anidulafungin and voriconazole is currently enrolling patients to investigate a possibly synergistic activity of this combination regimen.

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Unexplained fever during prolonged neutropenia: scientific background and study rationale

Part II deals on the treatment of fever in patients with prolonged neutropenia. Recommendations in current guidelines to continue broad-spectrum antibiotic treatment for 7 up to 14 days are based on the assumption that most febrile episodes in these patients are caused by bacterial infections, which can be rapidly fatal if not promptly treated.[62] These guidelines were predominantly derived from experience with patients with hematological and lymphoproliferative malignancies. However, fever may just as well be non-infectious. One should also realize that bacteremia accounts at maximum for 35% of infections among patients with hematological malignancies and fever, which is even less among patients with solid tumors.[5,63] Besides, no convincing data exist that neutropenic patients in whom evidence of a bacterial infection is lacking, will benefit from prolonged therapy. One might therefore argue that it would be sufficient to administer the empirically started antimicrobial therapy shorter than the recommended 7 to 14 days in patients with prolonged neutropenia, provided that a documented bacterial infection is lacking.

Historically, reflections on how to manage fever during prolonged neutropenia have been part of an evolving landscape during the preceding decades. The first therapeutic trial performed by the EORTC in 1978 showed a mortality rate of above 20% in patients with gram-negative bacteremia and ~15% in patients with sepsis due to gram-positive microorganisms.[64] Even worse were numbers of an earlier study from 1962, in which the mortality rate approached up to 90% in patients with severe underlying disease and gram-negative bacteremia.[65] However, the outcome of bacteremia in neutropenic patients has continued to improve since. In a recent study, the 30-day overall mortality was observed to be 10% and 6% for patients with gram-negative and gram-positive bacteremia, respectively.[66] However, it should be noted that not all studies report such

reassuring numbers, which might be partly explained by differences in study populations.[63,67]

The universally applied strategy of the immediate initiation of empirical broad-spectrum antimicrobial therapy in case of fever in neutropenic hematology patients is likely to be responsible for this impressive decrease in mortality.[68,69] This policy has been incorporated in standard practice since the high mortality of bacteremia in patients with deep neutropenia was documented. The antibiotic regimens that have been used for this purpose are diverse. Also, the preferred agents have changed due to the documented shift in the epidemiology of gram-negative to gram-positive microbes, as outlined in more detail in the general introduction on this thesis.[70] In many centers, vancomycin for gram-positive coverage used to be part of the empirical antibiotic treatment for neutropenic fever. However, this was abandoned later as randomized clinical trials failed to demonstrate an improved outcome when vancomycin was added to broad-spectrum beta-lactam antibiotics.[71] Currently, all hospitals have their own initial antibiotic regimens for patients with neutropenic fever, based on the local epidemiology and antibiotic susceptibility patterns of bacterial isolates.

Another important issue that should be addressed is the duration of antimicrobial therapy of febrile neutropenic patients. Distinctions should be made between patients with or without documented infection, prolonged deep neutropenia, and temperature response after the start of antimicrobial therapy. Obviously, the decision to stop treatment is more difficult to make in patients with prolonged neutropenia and ongoing fever despite initial broad-spectrum antimicrobial therapy but without documented infection.

Not surprisingly, different opinions exist and various strategies were implemented in different centers. In a randomized controlled trial published in 1982, 50 febrile neutropenic (neutrophil count, <500 cells/µl) patients, in whom initial evaluation did not demonstrate an infectious etiology after 7 days, were randomized to stop treatment or to continue the empirically started antimicrobial therapy with or without the addition of an antifungal agent until recovery from neutropenia and defervescence had occurred.[72] Clinical or microbiological infection was subsequently documented in 9 of 16 patients who discontinued initial antibiotic therapy, in 6 of 16 patients who continued their initial empirical treatment without addition of amphotericin B, and in 2 of 18 patients who continued the empirical treatment with addition of amphotericin B. These data paved the way for the addition of empirical antifungal treatment in case of persisting neutropenic fever. In case of bacteremia, other experts favored an early change from the initial therapeutic regimen to a strategy aimed at secondary prophylaxis in patients with persistent neutropenia and fever, e.g., fluoroquinolone-prophylaxis after occurrence of gram-negative bacteremia.[73,74]

Current guidelines from the Infectious Diseases Society of America (IDSA) advocate the continuation of initial broad-spectrum therapy for 2 weeks in febrile patients with prolonged neutropenia.[62] According to these recommendations, one might consider to stop empirical treatment if no bacterial infection is documented after careful reassessment, provided that the general condition of the patient is stable. However, these guidelines date from 2002. At those days, the administration of routinely antimicrobial prophylaxis, as currently applied in many modern centers, was not implemented in standard treatment protocols.

The administration of non-absorbable antimicrobials with the intention to selectively decontaminate the gram-negative microorganisms from the gut, aimed at prevention of bacterial infections in neutropenic patients, came into use in the eighties. Indeed, the gut is the most important reservoir of these potentially pathogenic microorganisms. This selective prophylaxis resulted in a significant decrease of severe infections.[75-77]

Gram-negative prophylaxis was subsequently expanded by the administration of components that were not only locally active in the gut, but were also absorbed and therefore systemically active as well. The main drug used for antibacterial prophylaxis in the 1980s was trimethoprim-sulfamethoxazole (TMP-SMZ).[74,78-80] The International Antimicrobial Therapy Project Group described the results of a randomized EORTC-study, published in 1984, on the use of TMP-SMZ in the prevention of infection during neutropenia.[78] Of 545 patients expected to develop prolonged neutropenia, and randomized to receive TMP-SMZ or placebo, data from a total of 342 patients were available for registration of infection and bacteremia. Infection occurred in 64 (39%) of 165 patients allocated to placebo and 46 (26%) of 177 patients randomized to TMP-SMZ, whereas bacteremia was noted in 32 (19%) and 22 (12%) patients, respectively. In the subset of 139 patients with AML, infection was observed in 35 (55%) of 64 placebo-treated patients and 31 (41%) of 75 patients in the TMP-SMZ group, whereas bacteremia occurred in 15 (23%) and 18 (24%), respectively. Differences were not significant. Gram-positive cocci were isolated less frequently from patients with bacteremia who were treated with TMP-SMZ, but more of their isolates were resistant to TMP-SMZ compared with those from patients allocated to placebo.

Some early studies have provided evidence that early discontinuation of antibiotic therapy that was empirically started for treatment of neutropenic fever may be safe under close observation and selected circumstances, including routine antimicrobial prophylaxis. A study published in 1984 described the results of 16 patients in a cohort of 429 patients with neutropenia and fever, in which the presence of an infectious etiology was considered unlikely after a careful assessment, despite ongoing fever.[81] The initial empiric therapy was therefore discontinued after a mean of 4.8 days. Of these patients,

14 received concurrent oral antibiotic prophylaxis with either non-absorbable drugs or systemically active compounds. Discontinuation of antibiotics proved to be appropriate in half of these patients. In the other 8 patients, antibiotics had to be reinstituted within a mean of 2.4 days. Another study reported on 52 adult hematology patients with a neutrophil count <500 cells/µl, who endured a total of 77 febrile episodes while receiving oral antibiotics (neomycin, polymyxin B and amphotericin B) as selective digestive decontamination.[82] Patients were not treated with empirical antimicrobial therapy at the onset of an episode of neutropenic fever if bacterial infection was considered unlikely (34 episodes). In 15 of these 34 episodes, no therapy was started at all; in the other 19 patients antibiotics were administered later on during their neutropenic episode. Overall, antibiotics were started in 62 cases of likely infection, including the 19 episodes mentioned above. In 40 episodes, therapy was discontinued after 72 to 96 hours because microbiological cultures remained negative and clinical evidence of an infection was lacking. Survival of these patients was 100%, and the survival rate during febrile episodes with confirmed infection was 85%.

Especially the administration of fluoroquinolones has proven to prevent gram-negative bacteremia effectively and even to reduce mortality.[83-86] In a multi-center, double-blind, randomized trial published in 2005, Bucaneve and colleagues described the benefits of levofloxacin prophylaxis in patients during neutropenia.[83] In this study, 760 adult patients with acute leukemia (stratified as high-risk) or solid tumors and lymphoma (stratified as low-risk) were randomized to receive prophylaxis with either levofloxacin or placebo. After assignment, 375 patients were treated with levofloxacin, and 363 patients received placebo. Patients were eligible if the expected duration of chemotherapy-induced neutropenia (neutrophil count, <1000 cells/µl) was more than 7 days. Prophylaxis until recovery from neutropenia was given 1 to 3 days before the administration of cytotoxic chemotherapy, or within 3 days before or after the re-infusion of stem cells in case of hematopoietic stem cell transplantation. The intent-to-treat analysis showed that fever was present in 65% of patients allocated to levofloxacin versus 85% of patients randomized to placebo. Furthermore, in the levofloxacin group, less microbiologically documented infections and less episodes of overall as well as gram-negative bacteremia were observed. All these differences were statistically significant, and data were similar for patients with acute leukemia versus patients with lymphoma or solid tumors. Mortality rates were equal for both groups.

In a randomized study, also published in 2005, comprising patients with lymphoma or solid tumors, similar observations were made for patients treated with levofloxacin versus patients allocated to placebo.[84] Fewer febrile episodes were documented in the levofloxacin group, as well as a lower incidence of overall bacterial infections. Besides,

a lower rate of hospitalization was required to treat infectious complications. Again, mortality rates were similar for both groups.

Two recent meta-analyses of randomized, placebo-controlled trials on antibiotic prophylaxis during chemotherapy-induced neutropenia concluded that the reduction in infection rates and mortality outweighs the extra costs and adverse events.[85,86] Importantly, another study suggests that this also seems to be true for the risk of developing resistance, as raised before.[87]

No consensus was reached during the development of the IDSA-guidelines for the use of antimicrobial therapy in febrile neutropenic patients.[62] Guidelines state that in some special cases prophylaxis may be considered for a critical period of time, provided that the potential for resistant organisms is appreciated and outweighed. The authors admit that their advices are in a sense paradoxical in view of data supporting the efficacy of antimicrobial prophylaxis, but that concerns about the development of drug-resistant microorganisms, as has been described before,[88] plus the fact that prophylaxis has not been shown to consistently reduce mortality has led to their final recommendations. Importantly, these guidelines refer to publications that were not more recent than 1999, which is well before the high-quality evidence in support of fluoroquinolone prophylaxis was published.

Management of fever during prolonged neutropenia: observational safety data

An essential feature of the antibiotic strategy for the treatment of neutropenic fever among hematology patients as developed in our hospital over the last 10 years is the use of antimicrobial prophylaxis, preferably with oral fluoroquinolones and fluconazole. This regimen is administered to all patients with an expected duration of neutropenia of at least 10 days. Colistin is added for the first 10 days of neutropenia to minimize the risk of developing fluoroquinolone resistance and to accelerate decontamination of the gut. Patients at high risk of gram-positive bacteremia from severe mucositis receive additional penicillin until recovery from mucositis. In case of a new-onset episode of neutropenic fever, initial broad-spectrum antimicrobial therapy with imipenem is started, which is tailored to directed therapy in case of documented microbiological infection. In contrast to current guidelines, the empirically started antibiotic therapy is discontinued in hemodynamically stable patients, irrespective of the temperature response if no bacterial origin is identified after a standardized diagnostic work-up of 72 hours. Renewed clinical assessment of patients occurs on a daily basis.

In a prospective observational study (Chapter 5), we assessed the safety of this strategy among 166 hematology patients who were followed during 276 distinct periods of therapy-induced prolonged neutropenia (mean duration, 20.5 days). Of all studied neutropenic episodes, 74 of 276 (27%) passed without fever. Of all patients, 137 (82.5%) had 1 or more febrile episodes during the remaining 202 periods of neutropenia. The mean duration of fever in 113 patients who had 1 febrile episode was 5.5 days and imipenem was prescribed for a mean of 4.7 days. Infection was documented in 48 of these episodes, while 64 episodes were due to unexplained fever and 1 episode definitively had a non-infectious etiology. In patients with unexplained fever, the mean observed duration of fever was 4.3 days and imipenem was administered for a mean of 3.7 days.

Several aspects need to be emphasized. First, the all-cause mortality in our study population was reassuringly low (6 of 166 patients, 3.6%), compared with other data reported in the literature. At least 1 and possibly 2 of these deaths were related to IPA, and were therefore of a non-bacterial infection. In the remaining 4 patients, an infectious etiology could not be documented. However, autopsy was not performed, so an infectious origin could not be excluded with absolute certainty. Second, in 1 patient a gram-negative bacteremia was documented during the administration of gram-negative antimicrobial prophylaxis. In this patient, no gram-negative microorganisms were detected in the preceding bowel surveillance cultures. The vast majority of bacterial infections were due to gram-positive microorganisms, which is in agreement with current epidemiological data.[5]

Possible directions for further research

To summarize, our strategy deserves consideration in a setting of antimicrobial prophylaxis, and may be seen as a cautious next step in the evolving field on the management of patients with fever during prolonged neutropenia. The major advantage of this restrictive antimicrobial treatment policy would be a substantial reduction of the use of broad-spectrum antibiotics, which may reduce the risk of resistance development to these drugs. It may also guarantee a more eager search for the real cause of fever, which could be falsely tempered when broad-spectrum antibiotic therapy is continued.

However, our observational study lacked a control group, as the described policy has become the standard of care in our hospital for approximately 10 years now. Therefore, the safety of this strategy should preferably be compared with the current standard of care of prolonged broad-spectrum antibiotic therapy in a multi-center, randomized, clinical trial.

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Diagnosis of catheter-related bloodstream infection: scientific background and study rationale

The intensive treatment of hematology patients requires a reliable vascular access as an essential feature of medical care. The increased use of the central venous catheter (CVC) for permanent bloodstream access partly explains the global rise in the incidence of gram-positive bacteremia. Catheter-related bloodstream infection (CRBSI) is one of the leading causes of healthcare-associated bacteremia, and is mainly caused by gram-positive microorganisms which colonize the human skin. Several aspects related to CRBSI are outlined in **Part III**.

The magnitude of the risk varies greatly with the type of device. In a large meta-analysis, the highest risk of CRBSI was observed for long-term tunnelled or cuffed CVCs, and non-cuffed hemodialysis catheters with a rate of 22.5 and 21.2, respectively, per 100 catheters.[89] However, these numbers changed remarkably after correction for the longer dwell time of central catheters, compared with peripheral catheters or other short-time devices. When expressed per 1000 catheter-days, the rate of CRBSI in patients with long-term CVCs was 1.6, which compares favorably with the rate as observed for arterial catheters (1.7), or short-term CVCs (2.7). Still, the CVC in its many forms is the source of a vast amount of CRBSI.[90-94]

CRBSI can occur after microbial colonization of the device itself or, to a lesser extent, by the infusion of contaminated infusate.[95] Microorganisms gain access to the exoluminal or endoluminal surface of the catheter, to which they adhere and eventually become incorporated into a biofilm, consisting of amorphous and fibrous material.[96,97] Subsequently, biofilm-derived bacteria can enter the bloodstream and cause CRBSI. Access to the exoluminal surface of the catheter occurs after microbial invasion of the percutaneous tract at the insertion site, which is addressed as exit site colonization. Exoluminal CRBSI is most common for intravascular devices with a dwell time of less than 10 days.[98-100] The second route of catheter colonization is caused by microbial contamination following manipulations with the catheter hub. Endoluminal CRBSI is probably predominant in patients with long-term cuffed or tunnelled CVCs.[101,102] The third mechanism that enables microorganisms to gain access to the catheter is hematogenous seeding to the implanted device from a remote source of local infection.

In any patient with bacteremia and a CVC, the possibility of a CRBSI should be considered. CRBSI will be obvious in patients with symptoms of systemic infection and inflam-

mation around the catheter insertion site or tunnel tract. Mostly, however, the diagnosis remains a major challenge, requiring microbiological evidence to assess the catheter as the source of bacteremia in order to prevent unnecessary removal of the CVC.[103,104] Obviously, attempts to diagnose CRBSI should ideally rely on conservative diagnostic tests, i.e., with the device left in situ.

The most accurate technique, which does not necessitate CVC removal, is based on simultaneously drawn quantitative blood cultures.[105,106] A strong suspicion of CRBSI arises when blood cultures obtained from the CVC reveal a ≥5-fold higher number of colony forming units (cfu) compared to peripheral cultures. In a meta-analysis of diagnostic studies, a pooled sensitivity of 93% and specificity of 100% was observed for long-term catheters [105]. However, this technique is labor-intensive and costly, and therefore not widely available. A more or less similar approach is to calculate the differential time-to-positivity (DTTP). Data have shown that an accurate diagnosis of CRBSI can be made if the blood culture obtained from the CVC becomes positive at least 2 hours earlier than a simultaneously drawn peripheral blood culture in a blood culture detector that continuously monitors for positivity.[107-109] However, DTTP is less reliable when patients are pre-treated with antibiotic therapy.[109]

Other catheter-sparing techniques, e.g., the acridin-orange leucocyte-cytospin test or the endoluminal brush for the specific detection of an endoluminal catheter infection are not widely used to diagnose CRBSI, mainly because they are labor-intensive for either the treating physician or the microbiological laboratory.[104]

Yet, a definitive diagnosis of CRBSI is in many cases established after removal of the catheter for catheter tip culture. Detection of microbial colonization of the catheter tip has demonstrated to correlate well with CRBSI in a recent meta-analysis.[110] If the microorganism detected on the catheter tip corresponds with the microorganism obtained from a peripheral blood culture in the absence of another source of bacteremia, a presumptive diagnosis of CRBSI can be made. One should realise that this method can confirm or reject a clinical suspicion of CRBSI only after catheter removal.

The most widely used diagnostic technique for catheter tip culture is the semi-quantitative roll plate method developed by Maki and colleagues, more than 30 years ago.[111] Obviously, this method can only detect exoluminal CRBSI. As endoluminal CRBSI is believed to predominate in long-term CVCs, the roll plate method may be less useful for long-term devices. Therefore, several techniques, such as vortexing, centrifugation and especially the sonication technique were developed. Theoretically, these techniques detect both exoluminal and endoluminal catheter colonization.[98,112-115] By vortexing, centrifugation or performing the sonication technique of the distal catheter segment, microorganisms are released from both the inside of the catheter lumen and the external surface.

A recent randomized, prospective, comparative study demonstrated the sonication technique to be less sensitive for short-term catheters.[116] In the subgroup of long-term CVCs (in this particular study defined as a catheter dwell time of more than 7 days), a non-significant slightly better diagnostic accuracy was observed for the sonication technique. Of note, the maximal gain of a technique which is able to detect also endoluminal colonization is indeed expected in patients with long-term devices.

Two techniques to detect catheter tip colonization: diagnostic yield and comparison

We performed a prospective, randomized study to compare the yield of the roll plate method and the sonication technique to detect catheter tip colonization in hematology patients with long-term tunnelled catheters (Chapter 6). We also assessed whether tip culture using the sonication technique, could be of additional diagnostic value in patients with clinical suspicion of CRBSI. Tip colonization was detected in 77 of 313 studied tunnelled CVCs (mean dwell time, 55 days). Both techniques were performed on each catheter tip in a randomized order. Tip culture was positive in 38 of 159 cases (24%) using the roll plate method, and in 23 cases (14%) performing the sonication technique in the subset of catheters on which the roll plate method was performed first. In the subset of 154 CVCs on which the sonication technique was performed first, a positive culture was recorded for 28 (18%) and 30 (19%) catheter tips for the roll plate method and the sonication technique, respectively. In conclusion, the quantitative sonication technique did not offer surplus value compared to the semi-quantitative roll plate method. For both techniques, a decrease in the diagnostic yield was observed if the concerning technique was performed after the other, which is in agreement with observations made in other studies.[117] In the subgroup of 89 catheters that were removed due to clinical *suspicion* of CRBSI, CRBSI was established for 40 cases. The diagnostic accuracy of both methods was equal and disappointingly low, no matter whether catheters were stratified according to dwell time or pooled data from both techniques were used (sensitivity 48%, and specificity 84% for pooled data).

Of the observed 40 episodes of bacteremia in which the catheter was eventually removed, 35 patients were pre-treated with antibiotics in a failed attempt to save the catheter. This strategy is commonly followed in hemodynamically stable patients, as the placement of a new tunnelled device is costly and not without the risk of complications. However, the administration of antibiotics in case of clinical suspicion of CRBSI in an attempt to save the catheter may be the main reason for the observed low diagnostic accuracy. Indeed, the negative impact of antimicrobial pre-treatment on the diagnostic yield of a culture of the catheter tip has already been demonstrated for short-term

catheters.[118] The use of lower detection cut-offs for tip colonization may improve sensitivity, as we observed for the roll plate method in our study. However, future studies to establish the usefulness and clinical relevance of this particular observation are needed. So far, only a positive test result may be helpful to establish the diagnosis (positive predictive value of 70% for combining the roll plate method and the sonication technique). Due to the low sensitivity, a negative tip culture result does not rule out CRBSI, which leaves clinicians without a sufficiently accurate diagnostic tool.

Prevention of catheter-related bloodstream infection: scientific background and study rationale

It is clear that *prevention* of CRBSI would be preferable. CRBSI is responsible for a prolonged hospital stay, increased treatment costs and, to a lesser extent, attributable mortality. Many preventive strategies that each address a specific aspect of catheter maintenance are available, although current evidence leaves many topics unresolved.

The most important preventive strategy is education and implementation of evidence-based guidelines of catheter insertion and maintenance, preferably by a dedicated nursing team using adequate hand hygienic measures during catheter manipulations. Effective preventive measures include the use of maximal sterile barrier precautions during CVC insertion, wearing sterile gloves, a long-sleeved sterile gown, a cap, and a mask, together with the use of sterile sheet drapes and chlorhexidine-containing antiseptics.[119,120]

Several measures were developed to prevent endoluminal catheter infection. Impregnation or coating of the catheter polymer surface with antimicrobial or antiseptic agents may reduce the formation of a biofilm on the catheter surface by reducing microbial adherence.[104,121,122] For *antiseptic* catheters, most prospective trials show reduced rates of catheter colonization, and a trend towards a reduction of CRBSI. Only 2 trials reported a statistically significant reduction in the rate of CRBSI.[123,124] However, a meta-analysis of 11 randomized, placebo-controlled, clinical trials (2603 CVCs) on the prevention of CRBSI with chlorhexidine-silver sulfadiazine-coated catheters showed a significant reduction of CRBSI (summarized OR 0.56; 95% confidence interval, 0.37-0.84).[122] The same observation was made for catheter microbial colonization, as a surrogate end point for CRBSI. A more recent meta-analysis, published in 2008, established these conclusions.[125]

Newer strategies include the use of impregnated catheter hubs or needleless catheter connectors.[126] In a study published in 1996, the results on the use of a new hub model were presented.[127] One of the components of this hub consisted of a plastic cylinder closed at both ends by latex rubber caps, which limited an antiseptic connection chamber, filled with 0.2 ml of 3%-iodinated ethanol. Of the 151 surgical patients and patients

admitted to the intensive care unit who were included, 15 (10%) developed CRBSI. The rate of CRBSI in the control patients, who received catheters that were equipped with standard connectors (n=73), was significantly higher compared to patients of the intervention group (n=78), who received the new connector system (16% versus 4%; P<.01). Moreover, the rate of culture-positive hubs without associated bacteremia was significantly higher in the control group. However, a subsequent randomized trial among surgical and ICU patients equipped with a non-cuffed CVC with an anticipated dwell time of more than 7 days, in which the same commercial hub device was used, failed to show any benefit.[128]

The use of needleless connectors, which have demonstrated to reduce the risk of needlestick injuries is somewhat controversial, as a paradoxically increased rate of CRBSI has also been observed.[129] However, other studies showed a reduction of CRBSI in favor of needleless devices when compared to standard hub connectors. In a non-blinded, prospective trial which included 230 patients treated with total parenteral nutrition, the incidence of CRBSI was 1.89% per catheter in patients with a closed-system connector system (n=106), compared with 12.1% per catheter in patients with a conventional luer-lock connector (n=124), which was a significant difference.[130] In a more recent study, comprising 278 multi-lumen CVCs of 243 patients admitted to the intensive care unit (mean dwell time, 9.9 days), the incidence of CRBSI was 0.7 and 5.0 per 1000 CVC-days in patients with or without needleless connectors, respectively (P=.03).[131] In another randomized, prospective trial, the use of a closed needleless hub device was demonstrated to be an independent protective factor of both catheter tip and hub colonization.[132]

Several prospective studies, including the before mentioned meta-analysis, reported a decreased risk of CRBSI in case of *antibiotic*-coated catheters, although until now only 1 device is FDA-approved.[104,125,133-137] Despite concerns, several studies failed to detect development of antimicrobial resistance.[133,134,136,137] The same concerns raise for antibiotic catheter locks, demonstrated to be another effective measure to prevent endoluminal CRBSI.[97,138-141] Therefore, current guidelines advise that antibiotic locks for the prevention of CRBSI should only be used when other measures to reduce the incidence of CRBSI all failed.[104]

In this perspective, the preventive use of an ethanol lock seems attractive, as ethanol is an effective antiseptic agent with a broad antimicrobial spectrum, especially at higher concentrations. Furthermore, the use of ethanol does not lead to antibiotic resistance and will therefore not compromise future antibiotic treatment. Also, it would be cheap and a universally available lock solution.

Several case reports and case series on the successful use of ethanol locks for the *treatment* of CRBSI in patients with long-term intravascular devices and persistent bactere-

mia have recently been published. However, none of these studies were randomized clinical trials, and therefore the role of ethanol locks for the treatment of CRBSI remains controversial.[142-144] Onland et al. showed the results of a retrospective study among pediatric oncology patients with tunnelled silicone CVCs or a totally implanted device.[144] A 70%-ethanol lock solution was instilled for 5 days in patients with ongoing CRBSI after more than 48 hours of antimicrobial therapy. The lock was given for 12 to 24 hours and subsequently removed. Fifty-one treatment episodes in 40 patients were studied. The total number of CVC-days was 8054 days, with an infection rate of 6.3 per 1000 CVC-days. No relapse within 30 days was observed in 88% (45 of 51 episodes). In another study, the lock solution was given for 4 hours to 19 patients; 15 of the 17 patients who finished the complete study protocol had no relapse of CRBSI and retained their tunnelled catheter for another 47 days.[142] Dannenberg et al. reported on the efficacy of a 74%-ethanol lock (dwell time 20 to 24 hours; lock solution was flushed through) in addition to systemic antibiotics for the treatment of CRBSI in children.[143] In this retrospective study, a relapse during the next episode of leukopenia was observed for 8 of 24 patients (33%), compared with 8 of 15 (53%) relapses among children treated with systemic antimicrobial therapy alone.

Even fewer observational studies have been published on the *preventive* use of an ethanol lock.[145-147] Maki et al. have reported on the successful use of a 25%-ethanol lock solution for the prevention of recurrent CRBSI in a patient dependent on home total parenteral nutrition.[145] Metcalf and colleagues described a TPN-dependent patient with Crohn's disease, who repeatedly suffered from CRBSI.[146] After treatment for a new episode, the prophylactic instillation of 3 ml of a 70%-ethanol solution in the catheter was started after each infusion with total parenteral nutrition, initially with a dwell time of 2 hours but eventually during the entire period between 2 infusions. No relapse was observed for more than 3 years, with the same device functioning properly. In a recent case series, Mouw and colleagues described 10 pediatric patients (26 CVCs, 3556 catheter days) with tunnelled silicone catheters for total parenteral nutrition, who were treated with a 70%-ethanol lock solution between infusions (range 4-14 hours).[147] Infection rates in 5 children of whom data were available from the period before initiation of lock therapy declined from 11.2 to 2.1 CRBSIs per 1000 catheter days. In a randomized, clinical trial, Sanders and colleagues recently observed a significantly reduced incidence of CRBSI after the administration of a daily 70% ethanol lock in hematology patients with a tunnelled CVC.[148] However, non-stringent definitions for CRBSI were used and the study sample size was limited.

Efficacy and safety of a 70%-ethanol lock for prevention of catheter-related bloodstream infection

In a randomized, prospective clinical trial, we studied the efficacy and safety of a daily administered 70%-ethanol lock solution on the prevention of CRBSI in hematology patients with a newly inserted long-term tunnelled CVC in place (Chapter 7). Contrary to all supportive evidence, no significant benefit of the use of an ethanol lock was demonstrated in our study. Although our study showed a 41%-reduction of endoluminal CRBSI in patients allocated to ethanol locks for CRBSI as expressed per 1000 CVC-days, this was not significant (P=.19). Also, the 3.6-fold reduction as observed in patients treated with ethanol locks who classified for endoluminal CRBSI according to the strictest definition was not statistically significant (2 of 226 versus 7 of 222; P=.103).

Non-severe adverse events leading to early discontinuation of study medication, like altered taste, facial flushing, and feelings of drowsiness were all significantly more common in patients allocated to ethanol. Major adverse events, e.g., mortality and occurrence of CVC thrombosis, were equally divided between both groups, which is in agreement with other data.[149] Whether the loss of integrity of 1 of 226 ethanol-instilled devices should be attributed to a causative effect of ethanol remains unclear. However, the same observation was reported in 1 of 26 CVCs in another study.[147] Another earlier study reported the qualitative softening of polyurethane catheters after sustained infusion with 70%-ethanol, although no significant damage of the catheter lumen was observed after scanning with an electron microscope.[150] On the contrary, the biomechanical and structural properties of both polyurethane and silicone catheters, submerged in an ethanol solution for 9 weeks, did not reveal any structural change in a more recent study.[151] As a concern, it has been reported that a 100%-ethanol lock solution applied in CVCs was associated with catheter occlusion.[152] Therefore, cautiousness is required when ethanol is used for prevention or treatment of CRBSI, and concentrations above 70% should not be used.

Possible directions for further research

Several factors may explain the lack of efficacy as observed in our study, which have been discussed in Chapter 7 in more detail. One of the main reasons may be that a lock-based intervention would reasonably prevent only *endoluminal* CRBSI. As the observed rate of exoluminal compared to endoluminal CRBSI in our study was higher than expected, our hypothesis that the rate of CNS bacteremia as observed in other studies would reflect mainly endoluminal CRBSI in case of long-term devices, may have been inaccurate in retrospect. In this view, the observed 3.6-fold reduction of strictly endoluminal CRBSI for

patients allocated to ethanol locks is reassuring, as is the 41%-reduction for endoluminal CRBSI when expressed per 1000 CVC-days. However, the increased rate of side effects combined with the overall low incidence of endoluminal CRBSI in this specific patient population may raise the question, even in case of a significant reduction of CRBSI, whether the clinical benefits of an ethanol-lock would outweigh the extra amount of effort, costs and patient discomfort.

Research should be prompted to focus also on non-CVC related CNS bacteremia, as several observations indicate that CNS colonization of airway or gastrointestinal mucosa and subsequent translocation during severe mucositis might also cause bacteremia.[153,154] We did genotypic identification of CNS in a random sample of 15 patients with documented bacteremia who had concomitant CNS in rectal and/or vaginal mucosa or mouth swabs. Identical CNS strains in blood and mucosa were identified in 6 of 15 patients (40%). Although the relevance of mucositis-associated bacteremia is not fully elucidated yet, one may hypothesize whether a next step to prevent CNS bacteremia could be the expansion of antimicrobial prophylaxis with oral, non-absorbable vancomycin for eradication of gastrointestinal CNS. However, important questions related to benefit-versus-harm aspects need to be answered first, including the risks of the development of vancomycin-resistant CNS and *Enterococcus faecium* strains.

In the mean time, additional studies on the use of a preventive ethanol lock solution should preferably be performed in patient populations with high incidence of (endoluminal) CRBSI, e.g., patients receiving long-term treatment with total parenteral nutrition.

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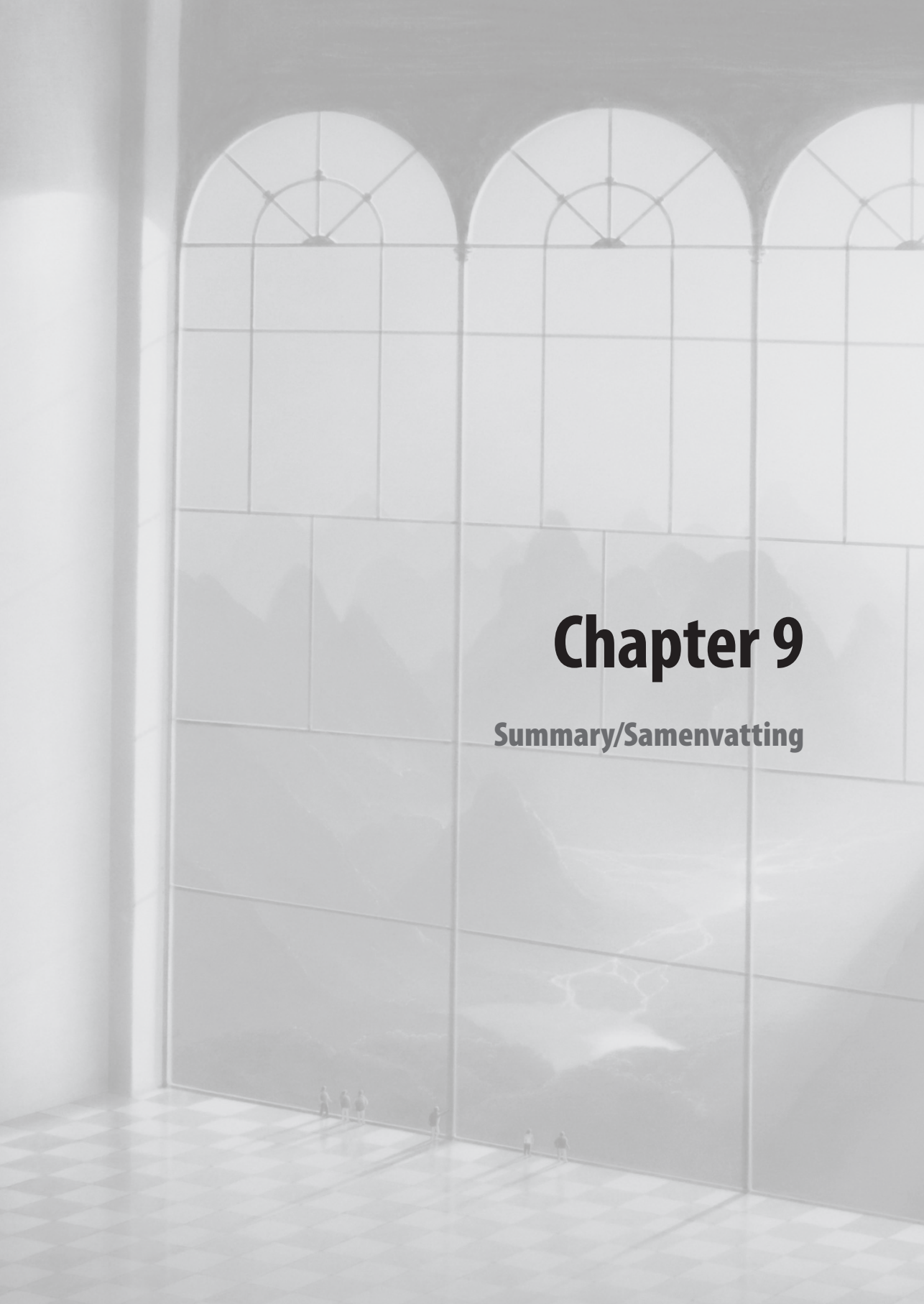

Chapter 9

Summary/Samenvatting

Summary

The innovative treatment strategies, developed during the second half of the 20th century to induce remission of acute leukemia, were life saving for many hematology patients. However, these intensive and aggressive modalities led to a treatment-induced disturbed immunologic defence as well as a disruption of the host's mechanical defence barriers against infectious organisms. Therefore, apart from the management of chemotherapy-associated toxicity or graft-versus-host disease, the management of the infectious complications associated with prolonged neutropenia, T-cell dysfunction and mucositis became a major new challenge. In current hematology, infectious complications represent a dominant cause of therapy-related morbidity and mortality. Thus, further improvement of survival will only be possible if the management of infectious complications caused by the anti-leukemic therapy is regarded as an integral part of patient care.

Reasonably, adequate *prevention* of infectious complications would mean a major step forward. The aim of the work as described in this thesis is to contribute to the development of such preventive strategies and their incorporation in daily patient care. An overview of the major developments in the hematological treatment of patients with acute leukemia is described in **Chapter 1**. This chapter also provides an outline of the infectious complications on which the work as described in this thesis has focussed.

Invasive fungal infections are an important source of mortality in patients receiving high-dose chemotherapy or immunosuppressive agents for hematological disease. In **Part I,** the results of 3 studies on the impact and prevention of invasive pulmonary aspergillosis (IPA) are presented. Because the inhalation of airborne *Aspergillus* conidia in the lungs is the first step in the pathogenesis of IPA, an inhaled formulation of an antifungal agent will attack the infection at the site of origin. Concurrently, inhalation of the drug will minimize systemic adverse effects.

Chapter 2 addresses the results of a randomized, placebo-controlled trial on the prevention of IPA. In this study, aerosolized liposomal amphotericin B or placebo was administered for two days a week during course-related neutropenia, using an adaptive aerosol-delivery system. The intent-to-treat analysis showed a significant and promising reduction of probable and proven cases of IPA in patients treated with aerosolized liposomal amphotericin B. We observed 18 IPA cases among 132 patients (14%) treated with placebo versus 6 IPA cases among 139 patients (4%) allocated to aerosolized liposomal amphotericin B (P=.005). Results for on-treatment analyses and secondary endpoints were equally encouraging.

Given these promising results, more extensive tolerability and safety data are needed. In **Chapter 3**, data on the tolerability, systemic toxicity and pulmonary function effects

of short-term (maximum duration, 6 weeks) nebulization therapy with aerosolized liposomal amphotericin B are presented, as observed in a random selection of all enrolled patients in the prophylaxis trial. Thirty eight patients (41 episodes) received liposomal amphotericin B, 39 patients (49 episodes) received placebo. No serious adverse events were observed, and only coughing was significantly more often reported in patients treated with aerosolized liposomal amphotericin B. No renal or hepatic toxicity was observed. Proportions of patients with a significant short-term decline in pulmonary function tests were equal in both groups. Twenty-six of 38 patients (68%) treated with liposomal amphotericin B versus 31 of 39 patients (79%) treated with placebo had no significant decline in pulmonary function during their entire prophylactic nebulization period, which is a non-significant difference. However, the considerable rate of patients in the placebo arm in whom a significant decline in pulmonary function was observed, probably also illustrates the limited value of a well-validated test during non-optimal test circumstances.

Because hospital resources are not infinite, reliable data on the diagnostic and treatment-related costs of IPA, and the outcome after infection are crucial to evaluate the cost-effectiveness of any future IPA-preventive strategy. In **Chapter 4**, data from an observational study among 269 patients, treated for acute leukemia during 2002 through 2007, are presented. Evidence of IPA was collected using high-resolution CT of the thorax and galactomannan measurement, preferably in bronchoalveolar fluid specimens. A total of 80 patients developed IPA; 48 (18%) had probable or proven infection, and 32 (12%) had possible IPA. The overall mortality rate was 26% in patients with IPA versus 16% in patients without IPA (P=.08), reflecting an IPA-attributable mortality rate of 10%. Diagnostic and therapeutic IPA-related additional costs, corrected for neutropenia duration, increased with €8360 and €15,280 for patients with possible and probable or proven IPA, respectively, compared with patients without IPA (P<.001). Therefore, effective prevention in patient populations with high IPA incidence has the potential to save lives and a substantial amount of resources.

Part II deals on the treatment of fever in patients with prolonged neutropenia. Current international guidelines recommend empirical treatment with broad-spectrum antimicrobial therapy for 7 to 14 days. However, the argumentation that prolonged empiric therapy in case of unexplained fever (UF) during neutropenia will result in a more favorable outcome does not take into account the efficacy of selective gram-negative antimicrobial prophylaxis on the prevention of bacteremia and mortality. Therefore, in our hospital, febrile patients initially receive empirical treatment with imipenem, which is discontinued if no bacterial etiology of the fever is identified after a standardized diagnostic work-up of 72 hours.

In **Chapter 5**, we report on the treatment of fever in a prospective observational study among hematology patients with prolonged neutropenia, who received antibiotic prophylaxis with fluoroquinolones and fluconazole. A total of 166 patients were evaluated during 276 neutropenic periods; 82.5% had ≥1 febrile episode. A total of 317 febrile episodes were observed, of which 177 (56%) were diagnosed as UF, while an infectious origin was documented in 135 episodes. The mean duration of all-cause fever in neutropenic periods with 1 febrile episode was 5.5 days, and the mean time of imipenem treatment was 4.7 days. In patients with UF, the mean duration of fever was 4.3 days, and imipenem treatment was given for 3.7 days. The observed overall mortality rate was 3.6% (6 of 166 patients); no patient died from untreated bacterial infection. Our study shows that discontinuation of empirically started imipenem for fever during neutropenia in hematology patients on continuous fluoroquinolone and fluconazole prophylaxis is safe, provided that there is no documented bacterial origin of the fever.

In **Part III**, we focus on another major infectious complication, the catheter-related bloodstream infection (CRBSI). The majority of the patients treated for hematological malignancies have permanent bloodstream access, which is warranted by an indwelling central venous catheter (CVC).

Diagnosing CRBSI still often involves a culture of the catheter tip. The conventional method is the semi-quantitative roll plate method. However, the use of the quantitative sonication technique could have additional value, as it may detect endoluminal microorganisms more easily. Because endoluminal infection is hypothesized to predominate in long-term CVCs, we compared both techniques for patients with long-term tunnelled catheters. The results of this randomized, prospective study are presented in **Chapter 6**. The overall tip colonization rate for 313 CVCs from 279 patients was 25%. Both techniques were performed in a random order. The diagnostic performance for the subgroup of patients with clinical suspicion of CRBSI (n=89) was limited and not different for both methods. Sensitivity and specificity were 45% and 84%, respectively, for the sonication technique versus 35% and 90%, respectively, for the roll plate method. No differences were observed after stratification according to dwell time. Antibiotic pre-treatment before catheter removal and tip culture, in an attempt to save the catheter, may partly explain this poor performance.

To some extent, endoluminal CRBSI can be prevented if an *antibiotic* solution is instilled in the catheter. However, the preventive use of antibiotics should be avoided if alternative options are available. For this purpose, ethanol is increasingly considered as a promising candidate. A major advantage of ethanol would be the broad antimicrobial spectrum without compromising future antibiotic treatment. **Chapter 7** addresses the results of a randomized, placebo-controlled trial on the efficacy and safety of a daily 70%-ethanol lock on the prevention of endoluminal CRBSI in hematology patients with

long-term tunnelled catheters. The intent-to-treat analysis was based on 448 CVCs, representing 27,745 catheter days. For ethanol locks, the incidence of endoluminal CRBSI per 1000 CVC-days was 0.70, compared to 1.19 for placebo. For endoluminal CRBSI according to the strictest definition, a 3.6-fold, non-significant, reduction was observed for patients receiving ethanol. More patients receiving ethanol discontinued lock therapy because of non-severe adverse events. As a result of the low incidence of endoluminal CRBSI in our study, the lack of statistical significance may partially reflect a lack of power. Alternative sources of bacteremia, like exoluminal CRBSI or microbial translocation during chemotherapy-induced mucositis may have been more important in our patients.

Chapter 8 represents a general discussion, in which the results of the various studies are discussed in more detail within their past and current scientific perspective. Also, possible directions for future research are provided.

Samenvatting

De innovatieve behandelstrategieën die gedurende de tweede helft van de 20e eeuw zijn ontwikkeld om remissie van acute leukemie te bewerkstelligen, zijn levensreddend geweest voor veel hematologiepatiënten. Echter, deze intensieve en agressieve behandelmethoden hebben onvermijdelijk tot een gestoorde immunologische afweer en een verstoring van de mechanische afweerbarrières tegen infectieuze micro-organismen geleid. Naast het beheersbaar maken van de toxiciteit die het gevolg is van een behandeling met chemotherapie en het omgaan met graft-versus-host gerelateerde problematiek ontstond derhalve een belangrijke nieuwe uitdaging op het gebied van het bestrijden van infectieuze complicaties die zijn geassocieerd met een langdurige neutropenieduur, T-cel dysfunctie en mucositis. Infecties zijn binnen de moderne hematologie verantwoordelijk voor een van de meest vóórkomende oorzaken van therapiegerelateerde morbiditeit en mortaliteit. Een verdere verbetering van de overlevingskansen van hematologiepatiënten is daarom ook alleen mogelijk als het beleid met betrekking tot infectieuze complicaties wordt gezien als een integraal onderdeel van de patiëntenzorg.

Logischerwijs zou adequate *preventie* van infectieziekten een belangrijke vooruitgang met zich meebrengen. Het in dit proefschrift beschreven onderzoek heeft ten doel om een bijdrage te leveren aan de ontwikkeling van dit soort preventieve maatregelen en aan een eventuele implementatie in de dagelijkse patiëntenzorg. In **Hoofdstuk 1** wordt een overzicht geschetst van enkele belangrijke historische mijlpalen in de behandeling van patiënten met acute leukemie. Ook wordt aandacht besteed aan de infectieuze complicaties waarop de in dit proefschrift beschreven onderzoeken zich hebben geconcentreerd.

Invasieve schimmelinfecties vormen een belangrijke doodsoorzaak in hematologiepatiënten die met hoge doseringen chemotherapie of andere immuunsuppressiva worden behandeld. In **Deel I** worden de resultaten van 3 studies met betrekking tot de invloed en preventie van invasieve pulmonale aspergillose (IPA) weergegeven. Omdat IPA zich in de longen kan ontwikkelen volgend op de inhalatie van in de lucht aanwezige *Aspergillus* conidia lijkt een poging tot preventie op dit niveau door middel van een geïnhaleerd antifungaal middel zinvol. Een bijkomend voordeel is dat inhalatie van het middel resulteert in slechts lokale activiteit waardoor eventueel systemische bijwerkingen worden geminimaliseerd.

In **Hoofdstuk 2** worden de resultaten beschreven van een gerandomiseerde, placebogecontroleerde trial gericht op preventie van IPA. Patiënten die deelnamen aan de studie werden tijdens de neutropene fase, die het gevolg is van de behandeling met chemotherapie, gedurende twee dagen per week behandeld met liposomaal amphotericine

B inhalaties danwel placebo, toegediend via een adaptief verneveltoestel. In de patiëntengroep die werd behandeld met liposomaal amphotericine B vernevelingen werd een aanzienlijke en veelbelovende reductie van IPA waargenomen. Onder de 132 patiënten die werden behandeld met placebo werden 18 gevallen (14%) van IPA waargenomen, vergeleken met 6 gevallen (4%) onder de 139 patiënten die werden behandeld met liposomaal amphotericine B vernevelingen (P=.005). Ook de on-treatment resultaten en de uitkomsten van de analyses van secundaire eindpunten waren bemoedigend.

Gelet op deze bevindingen is het van belang om in meer detail te letten op hoe het vernevelen met liposomaal amphotericine B door patiënten wordt verdragen, evenals op het feit of preventieve therapie hiermee veilig is. In **Hoofdstuk 3** worden data gepresenteerd die betrekking hebben op de tolerantie, systemische toxiciteit en mogelijke bijeffecten op de longfunctie van kortdurende (maximaal 6 weken) liposomaal amphotericine B vernevelingen. De hiervoor genomen steekproef uit het totaal aantal patiënten die deelnamen aan de profylaxestudie bestond uit 38 patiënten (41 episodes) die werden behandeld met liposomaal amphotericine B vernevelingen en 39 patiënten (49 episodes) die placebo kregen toegediend. Er werden geen ernstige bijwerkingen waargenomen; slechts hoesten werd significant vaker gerapporteerd in de groep die vernevelde met liposomaal amphotericine B. Evenmin was er een nadelig effect op de leverfunctie en nierfunctie. Het percentage patiënten bij wie kortdurend een significante vermindering van de longfunctie werd gezien, was niet verschillend tussen beide groepen. In de groep die vernevelde met liposomaal amphotericine B werd in 26 van de 38 patiënten (68%) gedurende de volledige vernevelperiode geen enkele daling van betekenis van de longfunctie waargenomen. Ditzelfde gold voor 31 van de 39 patiënten (79%) die werden behandeld met placebo. Dit verschil is niet statistisch significant. Daarbij is het aanzienlijke percentage patiënten in de placebogroep bij wie kortdurend een significante verslechtering van de longfunctie werd waargenomen naar alle waarschijnlijkheid tevens een illustratie van het feit dat ook een goed gevalideerde test van een slechts beperkte waarde is onder niet-optimale testomstandigheden.

Omdat ziekenhuisbudgetten niet ongelimiteerd zijn, is het van belang om een betrouwbaar overzicht te hebben van de kosten die samenhangen met zowel de diagnostiek als de behandeling van IPA, en om geïnformeerd te zijn over de outcome van een infectie. Mede aan de hand hiervan kan de kosteneffectiviteit van een eventuele preventieve strategie worden bepaald. In **Hoofdstuk 4** worden de resultaten gepresenteerd van een observationele studie die 269 patiënten omvat, die van 2002 tot en met 2007 werden behandeld voor acute leukemie. IPA werd gediagnosticeerd met behulp van een high-resolution CT van de thorax en het bepalen van het *Aspergillus* antigeen (galactomannan), bij voorkeur uit materiaal afkomstig uit een bronchoalveolair spoelsel. In totaal werd IPA gediagnosticeerd bij 80 patiënten; 48 patiënten (18%) voldeden aan de criteria voor een waarschijnlijke of bewezen infectie en 32 patiënten (12%) hadden

een mogelijke infectie. De overall mortaliteit bedroeg 26% in patiënten met, versus 16% in patiënten zonder IPA (P=.08), wat een attributieve mortaliteit van 10% ten gevolge van IPA impliceert. De voor de duur van de neutropene episode gecorrigeerde diagnostische en therapiegerelateerde additionele kosten bedroegen €8.360 en €15.280 voor patiënten met een mogelijke, respectievelijk waarschijnlijke of bewezen infectie in vergelijk met patiënten zonder IPA (P<.001). Effectieve preventieve maatregelen in een populatie waarin sprake is van een hoge incidentie van IPA lijken derhalve in potentie zowel levensreddend als kostenbesparend.

In **Deel II** staat de behandelduur van koorts bij patiënten met langdurige neutropenie centraal. De huidige internationale richtlijnen bevelen aan om gedurende 7 tot 14 dagen met breedspectrum antibiotica te behandelen. Echter, het argument dat een langdurige empirische behandeling van koorts zonder focus, optredend tijdens neutropenie, een betere uitkomst tot gevolg heeft, gaat voorbij aan de effectiviteit van selectieve gramnegatieve antibiotische profylaxe op het terugdringen van bacteriëmie episoden, met zelfs een gunstig effect op de mortaliteit. Het beleid in het Erasmus MC is derhalve om patiënten initieel empirisch te behandelen met imipenem, een breedspectrum antibioticum. Dit wordt gestaakt als er na een gestandaardiseerde work-up van 72 uur geen bacteriële etiologie kan worden vastgesteld.

In **Hoofdstuk 5** worden de resultaten besproken van een prospectieve, observationele studie die werd verricht bij hematologiepatiënten met een langdurige neutropenieduur die gedurende deze fase routinematig antibioticaprofylaxe kregen, bestaande uit fluoroquinolonen en fluconazol. In totaal werden 166 patiënten geëvalueerd gedurende 276 neutropenie perioden. Van de patiënten had 82.5% ≥1 koortsepisode. In totaal werden 317 koortsepisoden waargenomen, waarvan er uiteindelijk 177 (56%) werden gediagnosticeerd als koorts zonder focus, terwijl er in 135 episoden een infectieuze origine werd vastgesteld. De gemiddelde koortsduur in alle neutropenie perioden die gepaard gingen met slechts 1 koortsepisode bedroeg 5.5 dagen, waarbij de behandelduur met imipenem gemiddeld 4.7 dagen bedroeg. Als alleen patiënten met koorts zonder focus in ogenschouw worden genomen, bedroeg de gemiddelde koortsduur 4.3 dagen en de behandeling met imipenem 3.7 dagen. De overall mortaliteit bedroeg 3.6% (6 van de 166 patiënten). Er was niemand die overleed ten gevolge van een onbehandelde bacteriële infectie. Deze studie toont aan dat het staken van empirisch gestarte breedspectrum antibiotica in geval van koorts gedurende een neutropene episode waarbij geen bacterieel infectiefocus kan worden aangetoond, veilig is binnen een setting van hematologiepatiënten die routinematig antibioticaprofylaxe krijgen in de vorm van fluoroquinolonen en fluconazol.

In **Deel III** valt het licht op een andere belangrijke infectieuze complicatie, de katheter-gerelateerde bacteriëmie. De meerderheid van de patiënten die worden behandeld voor hematologische maligniteiten beschikt over een permanente toegang tot de bloedbaan in de vorm van een centraal veneuze katheter.

De diagnostiek van kathetergerelateerde bacteriëmie omvat veelal een kweek van de kathetertip. De conventionele tipkweekmethode is de semikwantitatieve rolplaatmethode. Echter, de kwantitatieve sonificatietechniek zou additionele waarde kunnen hebben als het gaat om de detectie van endoluminele micro-organismen. Omdat endoluminele katheterinfecties worden verondersteld te predomineren in geval van een katheter die voor lange(re) termijn wordt geplaatst, werden beide technieken onderling vergeleken in een patiëntenpopulatie met een getunnelde katheter voor langdurig gebruik. De resultaten van deze gerandomiseerde, prospectieve studie worden gepresenteerd in **Hoofdstuk 6**. Het overall percentage van microbiële kolonisatie van de kathetertip in 313 centraal veneuze katheters, afkomstig van 279 patiënten, bedroeg 25%. Beide technieken werden uitgevoerd in een willekeurige volgorde. De diagnostische waarde van het kweken van de kathetertip in de subgroep van 89 patiënten bij wie sprake was van een klinische verdenking op een katheterinfectie bleek laag en niet verschillend voor beide methoden. De sensitiviteit en specificiteit van de sonificatietechniek bedroeg respectievelijk 45% en 84%, vergeleken met 35% en 90% voor de rolplaatmethode. Er was evenmin een verschil als katheters werden gestratificeerd op basis van de tijdsduur die ze in de bloedbaan hadden gezeten. Mogelijk wordt de opbrengst van beide technieken negatief beïnvloed door het toedienen van antibiotica in geval van een klinische verdenking op een katheterinfectie, in een poging om de centraal veneuze katheter te sparen.

Tot op zekere hoogte kan een endoluminele katheterinfectie worden voorkomen door de instillatie van een antibiotica oplossing in de diverse lumina van de katheter. Echter, het op een dergelijke manier preventief gebruik maken van antibiotica dient te worden vermeden als er alternatieve opties beschikbaar zijn. Ethanol wordt in dit verband gezien als een veelbelovende kandidaat. Een belangrijk voordeel van ethanol is het brede antimicrobiële spectrum, terwijl het eventueel toekomstige behandeling met antibiotica niet in de weg staat. In **Hoofdstuk 7** worden de resultaten beschreven van een gerandomiseerde, placebogecontroleerde trial naar de effectiviteit en veiligheid van een dagelijks toegediend 70%-ethanol lock op het voorkómen van een endoluminele katheterbacteriëmie in een populatie hematologiepatiënten met getunnelde centraal veneuze katheters. De analyse is gebaseerd op 448 katheters die in totaal 27.745 katheterdagen vertegenwoordigen. De incidentie van endoluminele katheterbacteriëmie in de groep patiënten die werd behandeld met een ethanol lock bedroeg 0.70 per 1000 katheterdagen, vergeleken met 1.19 in de groep die werd behandeld met placebo. Dit verschil is niet significant. Als de meest strikte definitie van een endoluminele katheterbacteriëmie werd gebruikt, werd een 3.6-voudige, niet-significante

reductie waargenomen in het voordeel van de patiënten die werden behandeld met een ethanol lock (P=.10). In deze laatste groep werd de behandeling significant vaker onderbroken of volgens een gemodificeerd schema gegeven ten gevolge van hinderlijke, overigens niet ernstige, bijwerkingen. Omdat in onze studie sprake was van een lage overall incidentie van endoluminele katheterinfecties kan het niet behalen van een significant verschil nog eventueel het gevolg zijn van een gebrek aan voldoende statistische power om een effect aan te tonen. Alternatieve oorzaken van episoden van bacteriëmie zoals exoluminele katheterinfectie of microbiële translocatie als gevolg van chemotherapie-geïnduceerde mucositis hebben wellicht een belangrijkere rol gespeeld in onze patiëntenpopulatie.

Hoofdstuk 8 omvat een algemene discussie, waarin de resultaten van de verschillende onderzoeken in meer detail worden bediscussieerd in hun historische en huidige wetenschappelijke perspectief. Tevens worden aanbevelingen gedaan voor mogelijk vervolgonderzoek.

Dankwoord

Het is me in de afgelopen jaren al een aantal keer gelukt om terug te komen op eerdere voornemens waarvan ik had aangenomen dat die toch redelijk vast omlijnd waren. Zo ook in dit geval: ik zou me nooit laten verleiden tot het doen van wetenschappelijk onderzoek, behalve dan eventueel een klein onderzoekje om toch een beetje mee te kunnen praten, iets wat dan vooral weinig (vrije) tijd in beslag zou mogen nemen. Toen de kans tot het uitvoeren van zo'n klein onderzoekje zich aandiende, bleek er helaas over de specifieke deelvraag toch net wat meer bekend dan aanvankelijk werd verondersteld. En ja, toen lag er toevallig nog wel iets anders op de plank wat er erg veelbelovend uitzag. En voor ik het besefte, zat ik er niet alleen tot over m'n oren in, maar merkte ik ook dat onderzoek doen, studies opzetten en waar nodig vlot trekken, data analyseren, manuscripten schrijven -en vooral herschrijven- niet een louter ontmoedigende bezigheid is, maar bij vlagen ook intrigerend en meeslepend is, je blikveld verruimt, je brengt op nooit vermoede locaties en tenslotte wellicht ook vormend is voor een aantal tot dan toe wellicht wat onderontwikkelde eigenschappen. Waarbij opgemerkt moet worden dat de steun van heel veel mensen, van vakgenoten tot aan het brede thuisfront, van heel uitgesproken tot meer op de achtergrond, van doorslaggevende betekenis is geweest voor het welslagen van deze onderneming. Dat bedenkend wordt het schrijven van een dankwoord met recht een *dankbare* taak.

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Curriculum vitae

De auteur van dit proefschrift werd geboren op 3 januari 1978 te Capelle aan den IJssel. Na het behalen van het atheneumdiploma in 1996 (Wartburg-college, locatie Revius, Rotterdam) studeerde hij geneeskunde aan de Universiteit Utrecht, alwaar hij in 2000 slaagde voor het doctoraalexamen. Zijn co-assistentschappen volgde hij aan de Erasmus Universiteit Rotterdam, alwaar in 2002 het artsexamen werd behaald. Na een jaar te hebben gewerkt als arts-assistent niet in opleiding tot specialist (ANIOS) in het Havenziekenhuis te Rotterdam, begon hij in september 2003 aan de opleiding tot internist. Gedurende de jaren hierna was hij werkzaam in het Erasmus MC (opleiders prof. dr. H.A.P. Pols en prof. dr. J.L.C.M. van Saase) en andermaal in het Havenziekenhuis (opleider dr. P.J. Wismans). In 2004 raakte hij betrokken bij klinisch onderzoek vanuit een samenwerking tussen de vakgroep Infectieziekten, de afdeling Medische Microbiologie en de vakgroep Hematologie van het Erasmus MC. Dit onderzoek nam gaandeweg toe in omvang en heeft uiteindelijk geleid tot de diverse bevindingen zoals beschreven in dit proefschrift. Vanaf 1 mei 2008 specialiseerde hij zich in het aandachtsgebied Infectieziekten (opleider dr. J.L. Nouwen). Op 1 januari 2010 vond de registratie tot internist-infectioloog plaats; sindsdien is hij werkzaam als internist in het Havenziekenhuis te Rotterdam.

List of publications

Slobbe L, Doorduijn JK, Lugtenburg PJ, el Barzouhi A, Boersma H, van Leeuwen WB, Rijnders BJA. Prevention of catheter-related bloodstream infection with a daily ethanol lock in hematology patients with tunnelled catheters: A randomized, placebo-controlled trial. PLoS One. 2010 (*in press)*.

Rijnders BJA, **Slobbe L**. Bronchoalveolar lavage fluid galactomannan for diagnosis of invasive pulmonary aspergillosis. Clin infect Dis. 2010;50(7):1070.

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Slobbe L, Polinder S, Doorduijn JK, Lugtenburg PJ, el Barzouhi A, Steyerberg EW, et al. Outcome and medical costs of patients with invasive aspergillosis and acute myeloid leukemia-myelodysplastic syndrome treated with intensive chemotherapy: An observational study. Clin Infect Dis. 2008;47(12):1507-12.

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Slobbe L, van Genderen PJ, Wismans PJJ. Two patients with ciguatera toxicity: A seafood poisoning in travellers to (sub) tropical areas. Neth J Med. 2008;66(9):389-91.

Sanderson JT, **Slobbe L**, Lansbergen GW, Safe S, van den Berg M. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1, and 19 in H295R human adrenocortical carcinoma cells. Toxicol Sci. 2001;61(1):40-48.

PhD portfolio

Name PhD student:	Leendert Slobbe
Erasmus MC Department:	Internal Medicine, section of Infectious Diseases
PhD period:	2004-2010
Promotor:	prof. dr. H.A. Verbrugh
Co-promotoren:	dr. B.J.A. Rijnders
	dr. J.K. Doorduijn

General academic skills

- Nederlands Tijdschrift voor Geneeskunde, Auteurscursus. Amsterdam, Nederland. 2005
- Minicursus methodologie van patiëntgebonden onderzoek en voorbereiding van subsidieaanvragen. Rotterdam, Nederland. 2009

In-depth courses

- Study-design, Netherlands Institute for Health Sciences (NIHES) course in Methodology. Rotterdam, The Netherlands. 2007
- Clinical Epidemiology, NIHES course in Clinical Epidemiology. Rotterdam, The Netherlands. 2007
- Classical Methods for Data-analysis, NIHES course in Biostatistics. Rotterdam, The Netherlands. 2007

Oral and poster presentations

- Safety of 70% ethanol as an antiseptic catheter-lock solution for tunneled polyurethane central venous catheters (CVC) in an ongoing randomized placebo controlled trial. 14th International Symposium on Infections in the Immunocompromised Host. Crans-Montana, Switzerland. July 2006 (poster presentation)
- Weekly aerosolized liposomal amphotericin-B to prevent invasive aspergillosis during prolonged neutropenia. Randomized placebo controlled trial. 46th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, USA. September 2006 (oral presentation, late-breaker)
- Alcohol-slot: verkapt alcoholisme of veelbelovende strategie? Aanwinsten in veneuze toegangswegen voor oncologie & hematologie. Studiedag Oncologische Heelkunde, UZ Leuven. Leuven, België. Maart 2007 (oral presentation)

- Outcome of early oral voriconazole therapy in 73 AML-MDS patients with invasive aspergillosis. 15th International Symposium on Infections in the Immunocompromised Host. Thessaloniki, Greece. June 2008 (poster presentation)
- Outcome and medical costs of 80 AML-MDS patients with invasive aspergillosis treated with oral voriconazole. 48th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, USA. October 2008 (poster presentation)
- Outcome and medical costs of 80 patients with invasive aspergillosis among 269 AML-patients treated with intensive chemotherapy. Wetenschapsdagen Erasmus MC. Antwerpen, Belgium. January 2009 (oral presentation)
- Three-day treatment with imipenem for unexplained fever during prolonged neutropenia. A prospective observational study in haematology patients receiving fluoroquinolone and fluconazole prophylaxis. 19th European Congress of Clinical Microbiology and Infectious Diseases. Helsinki, Finland. May 2009 (poster presentation)

Seminars and workshops

- Lijninfecties en de IC, hemato- of oncologiepatiënt: Zelfde probleem, andere aanpak? Avondsymposium Infectieziekten. Rotterdam, Nederland. Februari 2006 (oral presentation)
- Advancing knowledge, improving outcome in the management of invasive fungal diseases. Istanbul, Turkey. November 2007
- Infectieziekten Symposium Amsterdam (XII). Amsterdam, Nederland. December 2007
- Management van ernstige infecties. Breda, Nederland. Januari 2008
- Catheter-related bloodstream infections: Prevention, outcome & treatment. Rotterdam, The Netherlands. March 2008

Other activities

- 7th Congress of the European Federation of Internal Medicine (EFIM). Rome, Italy. May 2008
- Post-EFIM courses in het kader van ontwikkeling van management- en bestuurlijke kwaliteiten. Nederlandsche Internisten Vereeniging. Utrecht, Nederland. 2008