Managing invasive aspergillosis: impact on health and personalized prevention or treatment strategies

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General introduction and outline of the thesis

INTRODUCTION

Invasive aspergillosis is an infection caused by fungi that are members of the genus *Aspergillus*. The genus consists of several hundred mould species. When these organisms are viewed under the microscope, a characteristic view of the hyphae and conidial head can be identified. Botanist and priest Pier Antonio Micheli was the first who described and named this organism in 1729 (1). He derived the name from the typical morphology when viewed under the microscope, as it reminded him to the likeness of a holy water sprinkler, or *Aspergillum* in Latin.

Aspergillus fungi are highly prevalent in the environment all over the world, with specific subtypes being more prevalent than the other according to the region. They are typically ubiquitous fungi that play an important role in breaking down organic matter (2). Additionally, some of them are applied in the food processing industry to aid in the fermentation of cheeses, soy sauce and other products. A small subset of *Aspergillus* fungi is capable of causing disease in humans or animals. In Europe and the USA, the most important causative agent of human invasive fungal infections is *Aspergillus fumigatus*.

Aspergillus fungi are able to cause a wide spectrum of disease in humans, colloquially called aspergillosis. The way the disease manifests itself is mostly dependent on host factors that predispose the individual to allergic reaction, colonisation or invasive disease. On one end of the spectrum, invasive aspergillosis (IA) affects patients with seriously impaired immune systems. Patient who are recipients of a haematopoietic stem cell transplant (HSCT), solid organ transplant, intensive chemotherapy or corticosteroids can develop IA within the short period of time during which the immunity is iatrogenically impaired. Additionally, some haematological malignancies such as acute myeloid leukaemia or aplastic anaemia are characterised by prolonged absence of sufficient numbers of functional granulocytes and can by itself make a patient prone to IA. The presentation of chronic pulmonary aspergillosis (CPA) is more indolent and usually develops during a longer period of time in which the patients does not exhibit symptoms of disease. Patients at risk have no or only mildly impaired immunity, but are more prone to colonisation of the pulmonary tissue by *Aspergillus* fungi due to an underlying lung disease such as pulmonary emphysema. Finally, some patients develop an allergic immune response against *Aspergillus* fungi, resulting in symptoms caused by bronchial hypersensitivity in the context of a disease known as allergic bronchopulmonary aspergillosis (ABPA) (3).

This thesis focusses on IA, the other clinical presentations of disease caused by Aspergillus fungi are not discussed.

Pathophysiology of invasive aspergillosis

IA is an opportunistic infection, normally occurring only in patients with an impaired immune system. The immune system of a healthy person is capable of preventing invasive disease by *Aspergillus* fungi, despite unavoidable exposure to the organism. Several parts of the healthy immune system work in conjunction to attain this.

As the fungal spores enter the lungs through the airways, the barrier function of pulmonary epithelium forms the first line of defence. Tight junctions in the epithelium prevent the hyphae from penetrating the tissue lining. When this barrier function fails, alveolar macrophages attempt to control the spread of *Aspergillus* fungi. Macrophages perform an important function in presenting the antigens of foreign pathogens to other mononucleated cells. Pattern recognition receptors, mainly present on dendritic cells, characterise the antigens and subsequently activate additional cellular components of the adaptive immune cascade. When CD4+ T-cells are activated by the presented *Aspergillus*-specific antigens, they are triggered to express clonal expansion and to differentiate to effector T-cells or memory T-cells. In immunity against fungal infection, the role of the T-helper 1 cell and the T-helper 17 cell play the most important roles. These T-cells produce cytokines (IFN-gamma, TNF-Beta and IL-2, IL-17, IL-21 and IL-22) to achieve targeted recruitment of neutrophil granulocytes (4, 5). The granulocytes are responsible for the eradication of the pathogen mainly via phagocytosis. Oxygen free radicals are formed during phagocytosis, causing further cytotoxic damage to the fungal conidia.

Patients with deep neutropenia or a severely decreased cellular immunity might not be able to mount an appropriate immune response. One or more links in the chain of the immunological response are missing in the patient with for example agranulocytosis or a shortage of functional T-cells. Additionally, specific genetic mutations and polymorphisms of soluble pattern recognition receptors such as pentraxin 3 have been linked to an increased risk of IA (6).

Patients at risk for invasive aspergillosis

IA most commonly manifests itself in the lung of a patient. The lungs are in direct contact with the ambient air, which naturally contains *Aspergillus* spores. The paranasal sinuses are also a preferred location for the same reason. If the fungi manage to employ angio-invasive growth, dissemination to any other internal organ can occur. Angio-invasive, disseminated aspergillosis is difficult to treat and carries a high mortality (3).

Historically, IA is an extremely rare infection that only occurred in patients with congenital conditions that impair the immune system or after trauma that either anatomically facilitates colonization or results in a high exposure to fungal spores. The first case report describing a patient with IA dates from the year 1842 (7). The patient suffered from a chronic pneumothorax hampering the immune system to effectively combat the infection in the lungs. In the century following this first publication, mostly incidental cases have been reported in literature (8).

In the last few decades, the number of patients surviving with a severely impaired immunity has increased, for an important part due to availability of antibiotics and advances in oncological care. Allogeneic haematopoietic stem cell transplantation has been a very successful method to cure patients of severe haematologic malignancy, but patients undergoing this procedure have to survive for a prolonged period of time in which the immune system is nearly non-functional. Patients that underwent allogeneic haematopoietic stem cell transplantation (HSCT) or are being prepared for this procedure with the help of cytotoxic agents (remission-

induction therapy) are considered to have the highest risk of developing IA (9). Almost all centres in the world now apply prophylactic strategies with antifungal drugs to diminish the risk of IA in high-risk groups (10, 11).

Diagnosing invasive aspergillosis

Diagnostic criteria

In the past few decades, IA has increasingly been recognised as a highly prevalent disease within high risk groups. In a patient who develops a fever after allogeneic HSCT, IA should always be considered to be a possible cause. Depending on the severity of disease, antifungal therapy could be initiated immediately. As an alternative, the clinician may prefer to first treat with an antibacterial drug. If the patient recovers, the cause of fever is suspected to be a bacterium; if no recovery occurs, an invasive fungal infection will be more likely and an antifungal drug can subsequently be initiated. A third option would have been to perform additional diagnostics to guide the clinician in making the correct diagnosis. Over the past decades, a trend favouring early diagnostics instead of empirical treatment can be observed in the management of IA.

These three different strategies of managing a suspicion of IA are all based on the perceived likelihood of the presence of IA. This perceived likelihood is dependent on many factors; e.g. host factors, lack of response to antibacterial treatment, local epidemiology, and the experience the clinician has with the treatment of IA.

When the first scientific studies were executed, the difficulty of establishing a definite diagnosis of IA posed a serious problem (12). In many cases in which IA has been suspected and treated, no proof was present to support the diagnosis of IA apart from the course of the disease under antifungal treatment. It is likely that a substantial proportion of patients did not have IA, and recovery of their illness has not been due to antifungal therapy but followed the natural course of e.g. a viral infection. To improve the validity and reproducibility of scientific studies, an international expert meeting has been appointed to write a set of diagnostic criteria to be applied within scientific studies. The EORTC/MSG group published these definitions in 2002 (12), with a more recent update in 2008 (13) to incorporate new epidemiological insights and advances in microbiological techniques.

Diagnostic certainty is categorised as possible, probable and proven. By definition, IA can only be diagnosed in a host who is at risk due to an impaired immune system. Broadly speaking, possible IA is diagnosed with imaging, probable IA with microbiological techniques and proven IA with a tissue biopsy.

After the implementation of these criteria within studies, many medical centers around the world have adapted this system of diagnostic likelihood in a clinical setting. Although these criteria were not developed for use within the clinic, the criteria could provide guidance in diagnosis and treatment of IA and have also found their way into international guidelines to be applied in clinical practice (10, 14-16).

Imaging

Usually, the first step for the clinician in the assessment of a patient with suspected IA, is to perform imaging of the organ that is suspected to be involved. A high resolution computed tomography scan (HRCT scan) can show radiological signs that are compatible with IA. Patients at risk for IA (who are considered possible hosts) are also at risk for numerous other infections. The broad differential diagnosis of a patient with fever, respiratory symptoms and impaired immunity makes it impossible to establish a definite diagnosis with imaging alone (10, 13).

Microbiology

Microbiological techniques that aid the clinician to identify the causative agent of disease in a patient are fundamental for choosing the correct treatment. To establish a probable diagnosis of IA, microbiological tools should support the presence of *Aspergillus* fungi. Biochemical markers, of which Galactomannan and 1,3B-d-glucan are the most important ones, can be identified in either blood or in material that is collected from within the lungs by performing a bronchoalveolar lavage (BAL-fluid). Culture of BAL-fluid can also show growth of fungi. The fact that it can take several days before growth can be observed is clearly a disadvantage. On the other hand, culture allows phenotypical testing for antifungal resistance. A more novel microbiological technique that aids in the diagnosis of IA is the polymerase-chain reaction (PCR) (17-20). This technique involves the mass multiplication and subsequent detection of the presence of fungal DNA. An advantage of this technique is the short time required and the possibility to check for resistance mechanisms on a genetic level. Unfortunately, the yield of both culture and PCR remains low and the clinician is still mainly dependent on the use of galactomannan assay to establish a probable diagnosis of IA (10, 21-24).

Pathology

A proven case of IA can only be established by demonstrating the fungus in a biopsy taken from the tissue involved. A lung biopsy can have severe side effects and the added benefit of establishing a certain diagnosis must be weighed against the risk of complications such as bleeding and pneumothorax. For this reason, proven cases of IA still make up a small minority, usually less than 5% of patients (9, 13, 25).

Shortcomings of the EORTC/MSG definitions of invasive aspergillosis

As mentioned before, the definitions have been developed with the goal to be applied in studies and have not been developed to provide clear clinical guidance. Furthermore, the revised definitions are currently more than 10 years old, resulting in a suboptimal integration of current diagnostic modalities in the definitions. Additionally, the definition of host factors can be too rigid to apply to non-typical hosts of IA that have recently been described in literature. For example, patients that are admitted to the Intensive Care to be treated for Influenza or

COVID-19 have shown to be at risk for IA (26, 27) without clearly fulfilling the requirements of the host factors as mentioned in the criteria.

Antifungal treatment for invasive aspergillosis

In general, antifungal therapy consists of different classes of drugs than the ones that are employed to fight bacterial, parasitical or viral infections. This is necessary due to the difference of the cell structure of fungi when compared to organisms belonging to the other kingdoms. The three most important classes of antifungal agents with anti-*Aspergillus* effectivity are triazoles, polyenes and echinocandins. Amphotericin B, belonging to the class of polyenes, was the first drug that was introduced to treat IA (28). In 2002, the triazole voriconazole was introduced, showing superior survival and lower rates of adverse events (29). Voriconazole has since been the first choice of therapy for IA. In the meantime, a new formulation of amphotericin B in liposomes has been introduced. Liposomal amphotericin B (LAmB) was better tolerated and showed superior efficacy when compared with conventional amphotericin B (30-32). No direct comparisons between LAmB and voriconazole have been published. LAmB is widely recommended as a second choice of therapy, for example in case of intolerance or insufficient effect of voriconazole (10, 15, 24, 33).

Recently, a new class of antifungals has being introduced. Olorofim is the first drug belonging to the new class of orotomides. This class targets the dihydroorotate dehydrogenase (DHODH) in the *de novo* pyrimidine biosynthesis pathway expressed within *Aspergillus* fungi and is a new mechanism of action that does not overlap with the currently available other drugs. Clinical experience with the drug is currently limited to experimental settings. The drug does show promise as it can potentially meet the need for a new drug that is well tolerated and can be applied to treat triazole-resistant IA (34-37). The table (table 1) shows an overview of different classes of antifungal drugs and their mechanism of action.

Development of resistance against triazoles

Rising numbers of resistance rates reported in the clinic pose a new problem in the management of IA. Shortly after the introduction of voriconazole in 2002, the first report of resistance was found in the Netherlands (38). The mass use of fungicides in agriculture has been linked to the development of resistance mechanisms in the environment (39). The Netherlands has a well-developed agricultural industry and widely employs triazole derivatives to protect the crops. In the last few years, reported resistance rates in the Netherlands vary between 7% and 15%, and are still increasing (40). Increased environmental pressure for selection of resistance genes could possibly increase this number even further. The distribution of resistance rates within the Netherlands is given in Figure 1. The map shows the data published in Nethmap 2020, an annually appearing report on antimicrobial resistance rates within the Netherlands (40).

Table 1. Different classes of antifungal drugs and their mechanism of action

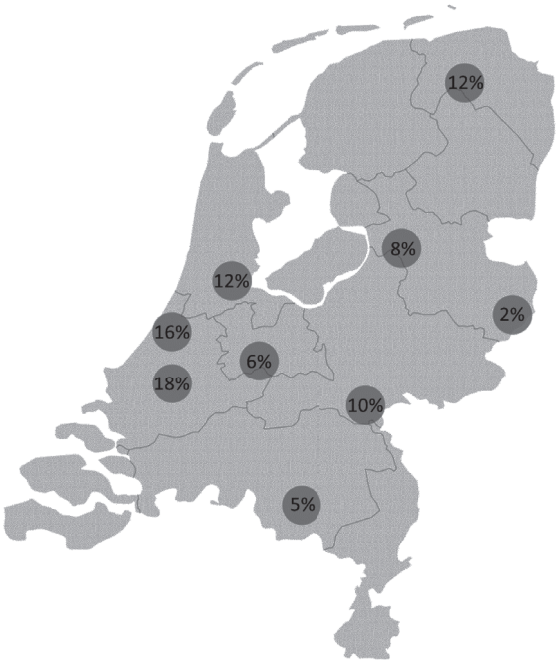
Class	Examples	Mechanism of action	Important safety considerations	Recommendation in guidelines	Resistance
Triazoles	Voriconazole Posaconazole Itraconazole Isavuconazole	Inhibitor of 14-alpha-demethylase, an enzyme necessary for the production of ergosterol, an important compound of the fungal cell wall.	Hepatotoxicity Visual hallucinations Important interactions with other drugs	First choice for primary treatment Contraindicated in case of demonstrated triazole resistance Not recommended when risk of triazole resistance is considered high	Common and increasing
Polyenes	Liposomal Amphotericin B	Binds directly to ergosterol	Hypokalaemia and other electrolyte disturbances Nephrotoxicity	Second choice for primary treatment First choice in case of demonstrated triazole resistance	Extremely rare
Echinocandins	Caspofungin Anidulafungin Micafungin	Inhibitor of an enzyme that produces 1,3 Beta-D glucan, an important compound of the fungal cell wall.	Serious side effects are uncommon	Sometimes recommended in conjunction with triazoles or polyenes	Rare
Orotomides	Olorofim	Inhibitor of dihydroorotate dehydrogenase (DHODH), an important compound in the *de novo* pyrimidine biosynthesis pathway	Insufficient data	Not yet implemented	Not yet reported

Impact of triazole-resistance on diagnosis and management of invasive aspergillosis

Triazole resistance complicates treatment of IA by losing a safe, effective drug in a disease that is already associated with a high rate of mortality despite adequate therapy. The impact of the problem is increased due to the difficulty of identifying a triazole-resistant or susceptible isolate. Susceptibility testing is only successful in about 10% of cases of IA (41). This means that for a large majority of patients, the clinically observed effect of treatment is the only indication of the presence of resistance. Furthermore, since triazoles are often employed in a prophylactic setting, clinicians might be inclined to forgo treatment with a triazole in a patient that received adequate triazole prophylaxis.

LAmB can be used as a drug of second choice but is hampered by the fact that renal toxicity can be severe and be a cause for need of treatment cessation (42, 43). Patients with triazole resistant IA who are intolerant of LAmB currently have no good alternative treatment option (33, 44).

Figure 1. Triazole resistance rates within 9 Dutch university hospitals and teaching hospitals in 2019.



Legend: Percentages represent the total share of analysed clinical isolates with resistance against one or more of the triazoles (itraconazole, voriconazole, posaconazole). The location of the circles corresponds to the relevant academic/teaching hospital, from left to right: Leiden University Medical Center, Erasmus Medical Center in Rotterdam, VU University Medical Center in Amsterdam, St Antonius Hospital in Nieuwegein, PAMM foundation (medical microbiological laboratories for four regional hospitals in Southeastern Brabant), Radboud Medical Center in Nijmegen, Isala Clinic in Zwolle, University Medical Center Groningen, and Medisch Spectrum Twente in Enschede. Data are derived from Nethmap/MARAN 2020

Another disadvantage of treatment with LAmB is the fact that it can only be administered intravenously. Patients often require therapy for 4 weeks or longer, and must usually remain in the hospital during intravenous therapy. Due to the risk of nephrotoxicity, it is recommended that kidney functions are monitored (43, 45-47). Due to the increasing rates of triazole resistance, more patients are being treated with LAmB.

Due to the increasing risk of triazole resistance and the fact that susceptibility testing is only successful in a minority of patients, Dutch national guidelines have recently advised to treat patients both with voriconazole and LAmB at the same time as combination therapy (14).

Mortality caused by invasive aspergillosis

Despite developments in antifungal drugs, the mortality resulting from IA is high (9, 48). To provide a better understanding of the way IA impacts mortality in patients with a haemato-oncological disease, the relation between the time of diagnosis and the time of death is important.

An important problem with the assessment of mortality in patient with IA is establishing attributability of IA to death (49, 50). Most studies present case fatality rates, usually describing the proportion of patients dying within 30 of 100 days after being diagnosed with IA. However, the population at risk for IA is subject to an important set of other factors that provide a higher risk of death, not related to IA (50). Factors that are a common cause of both IA and death are abundant within this population. For example, patients in whom leukaemia relapsed after allogeneic HSCT have a substantially increased risk of developing IA (51-53). Apart from the risk of IA however, relapsed leukaemia is prognostically very unfavourable and these patients are ultimately very likely to die from their malignancy (54). Several authors have made valuable contributions to defining a proper method to attribute mortality to IA (49, 50, 55).

OUTLINE OF THE THESIS

In the preceding paragraphs, the modern challenges within the management IA have been described. In this thesis, several of these challenges are explored more in-depth, and strategies to help circumvent or cope with these challenges are provided. Application of new diagnostic techniques and personalised treatment and prevention strategies can help us to rationally use the antifungal drugs we have currently available. A rational value-based policy of antifungal stewardship is necessary to manage the problems caused by triazole resistance and by the growing population at risk for IA.

Chapter 2 describes a meta-analysis of all currently available literature on the incidence of IA in patients treated for haematological malignancies. This group is considered to have the highest risk of IA and many preventive strategies are applied in this population. Additionally, the reported case fatality rates in these populations are presented.

Chapter 3 explores the contribution of individual risk factors associated with the development of IA.

Chapter 4 describes the survival rates of patients with IA in the Leiden University Medical Center in a time-dependent approach, to further explore the temporal relation between IA and death.

Chapter 5 explores the implications of rising resistance levels for the treatment of IA, and the added benefit of rapid identification of the presence of triazole-resistance using the PCR technique.

Chapter 6 describes a case series of 18 patients treated with LAmB after dismission from the hospital. The number of patients that need prolonged treatment with LAmB is increasing due to triazole resistance. However, experience with this treatment in an outpatient setting is limited because of concerns about toxicity and logistical challenges of intravenous drug administration outside of the hospital.

Chapter 7 shows the results of the implementation of a treatment strategy for IA that aims to avoid combination therapy with LAmB and voriconazole, while still minimizing the negative effect of potentially starting ineffective treatment. Additionally, a method to define mortality attributable to IA is described and applied in patients within the Leiden University Medical Center.

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Chapter 2

The burden of invasive aspergillosis in patients with haematological malignancy: a meta-analysis and systematic review.

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ABSTRACT

Objectives Successful treatment of haematological malignancies is hampered by invasive aspergillosis (IA), a life-threatening fungal infection that occurs in at least 10% of haemato-oncological patients all over the world. Case fatality rates (CFR) may fluctuate over time, depending on host pathogen interactions as well as treatment and quality of patient care. We conducted a systematic review and meta-analysis of current - i.e. 2008-revised EORTC-MSG criteria era - incidence and CFR of IA in patients with a haematologic malignancy.

Methods A systematic search was performed to identify all literature reporting populations with a haematologic malignancy and the incidence of IA, defined according to the EORTC/MSG 2008 criteria. Pooled cumulative incidences and CFR within 100 days were estimated using a random effects model for predefined patient populations and stratified by antifungal prophylaxis use.

Results The systematic literature search yielded 1285 publications of which 49 met the inclusion criteria. Overall, 16.815 patients were involved of which 1056 (6.3%) developed IA. Incidence of IA ranged from 4% (during remission-induction, with prophylaxis) and 11% (during remission-induction, without prophylaxis). Use of antifungal prophylaxis was associated with a lower rate of IA, most prominent in the pre-HSCT population. The pooled CFR within 100 days was 29% (95%CI: 20% – 38%).

Conclusions This study confirms that IA poses a relevant threat in the treatment of haematologic cancer worldwide despite the universal use of antifungal prophylaxis. These outcomes inform scientists and other stakeholders about the current burden of IA and may be used globally to direct, implement and improve antifungal stewardship programs.

INTRODUCTION

As an increasing number of patients survive with chronic or temporary conditions that compromise the immune system, the population at risk for invasive aspergillosis (IA) increases steadily. The advances in antileukaemic and antifungal therapy have contributed to an increased incidence and a decreased mortality risk of IA over time respectively (1-3). The increasing number of patients at risk has been mitigated by the implementation of different strategies to prevent IA (4). Guidelines concerning the use of antifungal prophylaxis, air filtration in haematology wards and pro-active diagnostic strategies have all been implemented in clinical practice. Emerging resistance of *Aspergillus* spp. against the triazoles, the class of antifungals most often used in prophylaxis and treatment of IA (5-8), forms a new challenge in managing IA.

New criteria for the diagnosis of IA have been published by the European Organization for the Treatment of Cancer in 2008 (9). The impact of these new criteria has been demonstrated in the analysis of historical cohorts and randomized controlled trials (RCTs) using the new criteria (10, 11). It is expected that this has influenced our view on the incidence and mortality of IA.

However, despite the developments in diagnostic strategies and prophylaxis regimens, estimations of the impact of IA in this population remain substantial (12). The incidence and mortality within patient populations treated for haematological malignancy is especially high due to their prolonged and severe immunocompromised status. Two treatment phases can be distinguished when assessing the impact of IA in patients treated with haematological malignancy: during remission-induction and consolidation chemotherapy and after allogeneic haematopoietic stem cell transplantation (HSCT). The reported incidence of IA ranges from 8% after allogeneic transplantation (13) to 12% before transplantation (1). Autologous transplantation yields a considerably lower risk (2%) (13).

Not only the incidence, but also the mortality rates differ according to the underlying condition. A systematic review about mortality rates according to the underlying condition has last been performed in 2001 by Lin et al (2), and it was found that case fatality rates (CFRs) are high (up to 88% in patients with haematological malignancy) and may differ according to the underlying condition. Current guidelines emphasise the importance of local incidence in the decision to use universal mould-active prophylaxis (14, 15). However, changes in CFR should also be taken into account when assessing the harm-benefit balance of prophylaxis.

To be able to make a rational choice for local measures to prevent and treat IA, it is critical to have knowledge of how institutional incidence and mortality rates relate to global rates. This necessitates an up-to-date analysis based on contemporary data. We therefore conducted a systematic review and meta-analysis to provide an up-to-date overview of the current incidence of IA in this patient population. Secondly, we performed a meta-analysis of the CFRs for all studies where this rate was available.

METHODS

Systematic literature review

We conducted a systematic literature review according to PRISMA guidelines (16) to identify RCTs and cohort studies reporting the incidence rates of IA in patients with haematological malignancies. A search of PubMed, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL) databases was performed on April 15th 2016. The search strategy is available in the supplemental data. We limited our strategy to studies in English. In addition, the references of key articles were searched to identify other eligible studies.

Eligibility criteria

The eligibility of a study was assessed according to the following items: (A) it was a cohort study or randomised controlled trial (RCT) (B) the population underwent remission-induction/ consolidation therapy or HSCT for haematological malignancy, (C) the number of included patients was >50, (D) incidence was reported or could be extracted or estimated from reported data, (E) IA diagnosis was classified as proven, probable or possible IA according to the revised 2008 EORTC criteria for the diagnosis of invasive fungal disease. Only proven and probable cases have been taken into account in the calculation of incidence and CFR. As the primary goal of the study was to evaluate the incidence rates, the report of CFR was not used as an inclusion criterion.

Data extraction

Studies were categorized according to underlying haematological disease, haematological treatment phase, antifungal prophylaxis and study method (cohort or RCT). Cumulative incidences and CFRs were extracted or calculated using the total number of patients, the total number of proven and probable IA, and the total number of deaths within 100 days of diagnosis of proven or probable IA. In case of a missing CFR in an included article, the corresponding author was contacted and requested for the additional data.

Risk of bias assessment

Several study characteristics that reflect risk of bias were assessed at the study level. Because it was expected that most eligible studies would be observational, the most important items that determine the risk of bias were assessed according to the Newcastle-Ottawa guidelines for observational studies (17). Most items that concern accuracy of the selection of the population at risk and outcome ascertainment have been included as selection criteria. Only the adequacy of follow-up remained to be appraised for each study. A proportion of lost to follow-up of >5%, or an unknown proportion, was considered a high risk of bias.

Statistical analysis

A meta-analysis based on risks of individual studies was performed using the STATA metaprop command (18). To increase the homogeneity of the different populations, four different categories of patient populations were defined for subgroup analyses. These categories are: during remission induction with (I) or without (II) mould-active antifungal prophylaxis, and after allogeneic HSCT with (III) or without (IV) mould-active prophylaxis. Only studies that reported the incidence rates of IA divided in discernible treatment groups of at least 25 participants were included in this subgroup analysis. To obtain the pooled CFRs, only studies that reported at least 10 cases of IA were considered. The data for all subgroup analyses were pooled at the aggregate patient data level. A random effects model was the most appropriate method to pool the results due to the expected clinical heterogeneity. All analyses were performed and figures were constructed using Stata Statistical Software: Release 12.1. College Station, Texas: StataCorp LP.

RESULTS

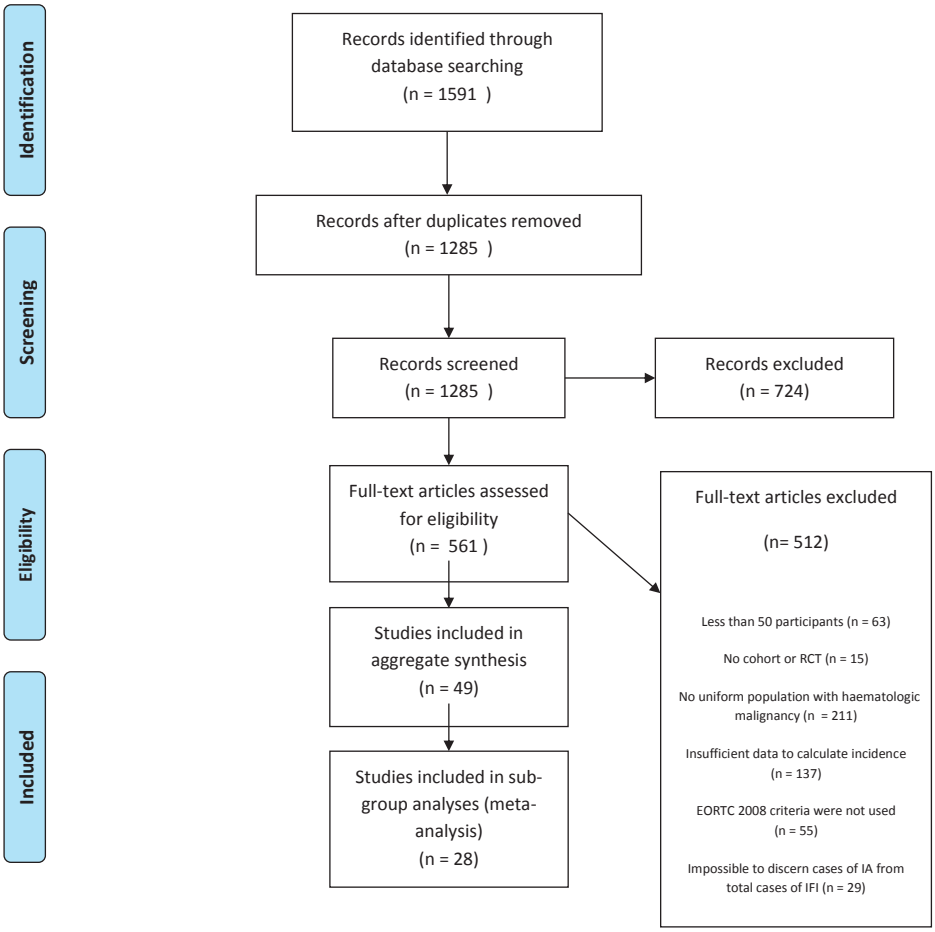
Total population

The systematic literature search yielded 1285 publications of which 49 met the inclusion criteria. Figure 1 shows the flowchart of the selection process. From the 49 publications, 68 distinct study populations were identified (table 1). From these 68 populations, 31 could be grouped according to the four categories we defined in the methods section: (I) 8 populations (19-24) during remission-induction therapy, with prophylaxis, (II) 8 populations (21, 23, 25-30) during remission-induction therapy, without prophylaxis, (III) 7 populations (31-37) after allogeneic HSCT, with prophylaxis, (IV) 9 populations (27, 32, 35, 38-43) after allogeneic HSCT, without prophylaxis. For the analysis of CFRs, 18 populations (23, 26-30, 32-34, 37-40, 44-46) were eligible for inclusion in the meta-analysis, of which 14 were also included in the meta-analysis of incidence rates. I-squared statistics for most analyses yielded high values with significant p-values, suggesting large heterogeneity between populations.

Summary statistics

Table 1 shows the characteristics of included studies that report the incidence of IA in patients with a haematological malignancy. Overall, 16.815 patients were involved of which 1056 (6.3%) were diagnosed with probable or proven IA. In 31 studies, describing 645 cases of IA, the CFR within 100 days was available; the crude aggregate amounts to 33%. These summary estimates are derived from a diverse population, with different prophylactic regimes and underlying disease.

Figure 1. Flowchart of literature selection



Risk of bias assessment

The adequacy of follow-up is shown in table 1. Most studies (n=32) did not report loss to follow-up rates but did exclude patients that failed to complete the entire treatment episode in the same hospital. Some studies report moderately high rates of loss to follow-up up to 9.8%. All studies with more than 5% of loss to follow-up were classified as "increased risk of bias". Egger's test for small-study effects yielded a p-value of <0.001 for the analysis of incidence rates and a p-value of 0.094 for the analysis of CFR. This indicates that a risk of publication bias may be present in the analysis of incidence rates.

Incidence

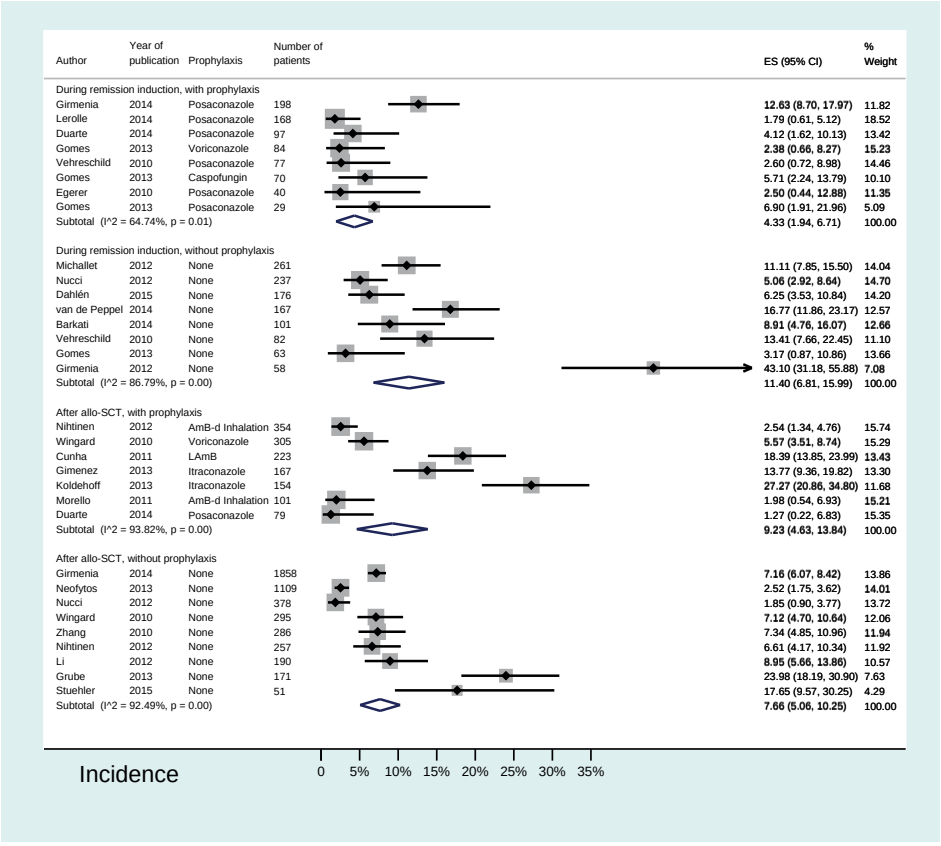
The meta-analysis of the IA incidence for the four subgroups is shown in figure 2. Statistical heterogeneity between studies was shown for all subpopulations. The pooled risks in our four subgroups are: (I) During remission-induction, with prophylaxis: 4% (95%CI: 2%-7%) (II)

During remission-induction, without prophylaxis: 11% (95%CI: 7%-16%) (III) After allogeneic HSCT, with prophylaxis: 9% (95%CI: 5%-14%) (IV) After allogeneic HSCT, without prophylaxis: 7% (5%-10%).

Case fatality rates

Figure 3 shows a forest plot of the CFRs within 100 days of the date of diagnosis of IA. The CFR varies clearly between studies, and ranged from 5% to 65%. The pooled CFR within 100 days for all 18 populations (reporting 535 cases of IA) is 29% (95%CI 20% - 38%). No evident difference between population categories was found. Six of our included studies (22, 24, 25, 27, 29, 32) had available information on the CFR of proven cases of IA, yielding a CFR of 78% in 27 proven cases of IA.

Figure 2. Meta-analysis of incidence of invasive aspergillosis in different subpopulations



Legend: Allo-SCT denotes allogeneic stem cell transplantation; ES estimate; CI confidence interval. The black dot represents the individual studies effect. The size of the grey squares represents the study weight according to the random effects model. The black lines represent the 95% confidence intervals of different studies. The diamonds represent the overall (or subgroup) effects, the outer edges of the diamonds represent the 95% confidence intervals.

Table 1. Characteristics of all included studies

Author	Study design	Country	Pub year	Study period	Prophylaxis
During Remission-induction therapy, with prophylaxis					
Gomes et al(21)	Cohort	Texas, USA	2014	2009-2011	Anidulafungin
Gomes et al(21)	Cohort	Texas, USA	2014	2009-2011	Caspofungin
Gomes et al(21)	Cohort	Texas, USA	2014	2009-2011	Micafungin
Duarte et al(20)	Cohort	Spain	2014	2007-2011	Posaconazole
Lerolle et al(22)	Cohort	France	2014	2007-2010	Posaconazole
Girmenia et al(19)	Cohort	Italy	2014	2007-2010	Posaconazole
Gomes et al(21)	Cohort	Texas, USA	2014	2009-2011	Posaconazole
Vehreschild et al(23)	Cohort	Germany	2010	2006-2008	Posaconazole
Egerer et al(24)	Cohort	Germany	2010	2006-2009	Posaconazole
Gomes et al(21)	Cohort	Texas, USA	2014	2009-2011	Voriconazole
Crude total					
During Remission-induction therapy, without prophylaxis					
van de Peppel et al (29)	Cohort	The Netherlands	2014	2005-2012	None
Barkati et al(25)	Cohort	Canada	2014	2008-2010	None
Gomes et al(21)	Cohort	Texas, USA	2013	2009-2011	None
Girmenia et al(30)	Cohort	Italy	2012	2006-2007	None
Michallet et al(26)	Cohort	France	2012	2004-2007	None
Nucci et al(27)	Cohort	Brazil(MC)	2013	2007-2009	None
Vehreschild(23)	Cohort	Germany	2010	2003-2005	None
Dahlén et al(28)	Cohort	Sweden	2016	2008-2013	None
Crude total					
After allogeneic SCT, with prophylaxis					
Morello et al(31)	Cohort	Italy	2011	1999-2009	AmBd inhalation
Nihtinen et al(32)	Cohort	Finland	2012	2001-2005	AmBd inhalation
Koldehoff et al(33)	Cohort	Germany	2013	2002-2012	Itraconazole
Giménez et al(34)	Cohort	Spain	2013	2005-2011	Itraconazole
Wingard et al(35)	RCT	USA (MC)	2010	2003-2006	Voriconazole
Duarte et al(36)	Cohort	Spain	2014	2007-2011	Posaconazole
Cunha et al(37)	Cohort	Italy	2011	2003-2010	LAmB
Crude total					

Study population	Notes	# pts	# IA cases	IA incidence	CFR within 100 days	CFR provided by author	Lost to follow-up
During RI-therapy	Not included in forest plot due to number of patients <25	18	1	5.5%	NA	NA	<5%
During RI-therapy		70	4	5.7%	NA	NA	<5%
During RI-therapy	Not included in forest plot due to number of patients <25	11	1	9.0%	NA	NA	<5%
During RI-therapy		97	4	4.1%	NA	NA	NA
During RI-therapy		168	3	1.8%	66%	NA	<5%
During RI-therapy		198	25	12.6%	44%	NA	NA
During RI-therapy		29	2	6.8%	NA	NA	<5%
During RI-therapy		77	2	2.6%	50%	Yes	None
During RI-therapy		40	1	2.5%	0	NA	NA
During RI-therapy		84	2	2.3%	NA	NA	<5%
		792	**45**	**5,7%**	**47% (in 30 cases of IA)**		
During RI-therapy		167	28	16.7%	39%	NA	None
During RI-therapy		101	9	8.9%	11%	Yes	NA
During RI-therapy		63	2	3.2%	NA	NA	<5%
During RI-therapy	Renovation work during study period	58	25	43.1%	36%	NA	NA
During RI-therapy		261	29	11%	31%	NA	NA
During RI-therapy		237	12	5.1%	33%	Yes	<5%
During RI-therapy		82	11	13.4%	27%	Yes	None
During RI-therapy		176	11	6.25%	9%	NA	9%
		1145	**127**	**11.1%**	**30% (in 125 cases of IA)**		
After allo-HSCT		101	2	2.0%	NA	NA	NA
After allo-HSCT		354	9	2.5%	55%	NA	NA
After allo-HSCT	Only AML patients	154	42	27.2%	21%	Yes	NA
After allo-HSCT		167	23	13.8%	13%	Yes	NA
After allo-HSCT		305	17	5.6%	NA	NA	None
After allo-HSCT		79	1	1.3%	NA	NA	NA
After allo-HSCT		223	41	18.4%	5%	Yes	NA
		1383	**135**	**9,8%**	**17% (in 115 cases of IA)**		

Table 1. Characteristics of all included studies (continued)

Author	Study design	Country	Pub year	Study period	Prophylaxis
After allogeneic SCT, without prophylaxis					
Girmenia et al(19)	Cohort	Italy (MC)	2014	2008-2010	None
Grube et al(39)	Cohort	Germany	2013	1998-2006	None
Neofytos et al(40)	Cohort	USA	2013	2000-2009	None
Li et al(41)	Cohort	China	2012	2000-2007	None
Nihtinen et al(32)	Cohort	Finland	2012	1996-2001	None
Nucci et al(27)	Cohort	Brazil (MC)	2013	2007-2009	None
Zhang et al(42)	Cohort	China	2010	2000-2007	None
Wingard et al(35)	RCT	USA (MC)	2010	2003-2006	None
Stuehler et al(43)	Cohort	Switzerland	2015	2012-2013	None
Crude total					
Other populations, with prophylaxis					
Lerolle et al(22)	Cohort	France	2014	2007-2010	Posaconazole
Barnes et al(56)	Cohort	UK	2013	2005-2009	Itraconazole
Cattaneo et al(57)	RCT	Italy (MC)	2011	2007-2009	Caspofungin
Chabrol et al(45)	Cohort	France	2009	2003-2006	Voriconazole
Chong et al(44)	Cohort	The Netherlands	2015	2005-2008	Itraconazole
Chong et al(44)	Cohort	The Netherlands	2015	2008-2012	Itraconazole + Aerosolized LAmB
Vehreschild et al(58)	Cohort	Germany	2014	2009-2011	Posaconazole and micafungin
Nachbaur et al(59)	Cohort	Austria	2015	2011-2012	Micafungin
Nicolle et al(60)	Cohort	France	2011	2004-2007	Posazonazole
Parody et al(61)	Cohort	Spain	2015	2003-2009	Mixed (66% voriconazole or posaconazole, 22% itraconazole, 11% Amb-d inhalation)
Springer et al (62)	Cohort	Austria	2016	NA	Mixed (micafungin and/or posaconazole and/or voriconazole, proportions unknown)
Takagi et al(63)	Cohort	Japan	2014	2006-2008	Voriconazole
Total					

Study population	Notes	# pts	# IA cases	IA incidence	CFR within 100 days	CFR provided by author	Lost to follow-up
After allo-HSCT		1858	133	7.1%	49%	NA	NA
After allo-HSCT		171	41	2.4%	29%	NA	NA
After allo-HSCT		1109	28	2.5%	43%	NA	NA
After allo-HSCT		190	17	8.9%	60%	NA	None
After allo-HSCT		257	17	6.6%	65%	NA	NA
After allo-HSCT		378	7	19%	0%	Yes	<5%
After allo-HSCT		286	21	7.3%	NA	NA	NA
After allo-HSCT		295	21	7.1%	NA	NA	None
After allo-HSCT		51	9	17.6%	22%	Yes	9.8%
		4595	**294**	**6,4%**	**44% (in 252 cases of IA)**		
With GvHD		96	0	0%	0%	NA	<5%
Mixed (during RI and after allo-HSCT)		549	53	9.8%	NA	NA	NA
During RI-therapy for AML, MDS or ALL		93	5	5.4%	0%	NA	None
During RI-therapy for AML or ALL	Renovation work during study period	88	3	4.5%	33%	NA	None
Mixed (during RI and after allo- or auto-HSCT)		108	12	9.4%	25%	NA	NA
Mixed (during RI and after allo- or auto-HSCT)		127	25	23.1%	8%	NA	NA
RI or SCT for different hem. mal.		106	1	0.9%	100%	NA	None
Mixed (during RI and after allo- or auto-HSCT)		100	2	2.0%	0%	NA	NA
During RI or after allo-HSCT for AML		1019	31	3.0%	NA	NA	NA
After allo-HSCT from unrelated donor		299	55	18.4%	NA	NA	NA
During RI for different HM and after allo- HSCT		84	4	4.8%	NA	NA	NA
After cord-blood HSCT		52	1	1.9%	0	NA	NA
		2721	**192**	**7,1%**	**24% (in 49 cases of IA)**		

Table 1. Characteristics of all included studies (continued)

Author	Study design	Country	Pub year	Study period	Prophylaxis
Other populations, without prophylaxis					
Aguado et al(64)	RCT	Spain (MC)	2015	2011-2012	None
Chabrol et al(45)	Cohort	France	2009	2003-2006	None
Erdmann et al(65)	Cohort	Germany	2016	2012-2013	None
Falantes et al(66)	Cohort	Spain	2014	2009-2012	None
Gheith et al(67)	Cohort	Tunisia	2015	2009-2011	None
Kim et al(68)	Cohort	SE-Asia	2012	2003-2009	None
Mendes(69)	Cohort	Brazil	2012	2001-2009	None
Nicolle et al(60)	Cohort	France	2011	2004-2007	None
Parody et al(61)	Cohort	Spain	2015	1997-2003	None
Pomares et al(70)	Cohort	Spain	2016	2007-2015	None
Rocchi et al (71)	Cohort	France	2014	2010-2012	None
Springer et al(62)	Cohort	Austria	2016	NA	None
Crude total					
Populations with unknown or mixed prophylaxis					
Reischies et al(72)	Cohort	Austria	2016	2014-2015	Unknown
Morrissey et al(73)	RCT	Australia, MC	2013	2005-2009	Mixed (37% itraconazole, 62% non-mould-active)
Kurosawa et al(46)	Cohort	Japan, MC	2012	2006-2008	Unknown
Kurosawa et al(46)	Cohort	Japan, MC	2012	2006-2008	Unknown
Kimura et al(74)	Cohort	Japan	2015	2007-2012	Mixed (38% itraconazole, voriconazole or micafungin, 62% non-mould- active)
Cattaneo et al(57)	RCT	Italy (MC)	2011	2007-2009	Mixed (83% mould-active azole, 17% no mould-active prophylaxis)
Loschi et al(75)	Cohort	France	2015	2003-2008	Unknown
Loschi et al(75)	Cohort	France	2015	2003-2008	Unknown

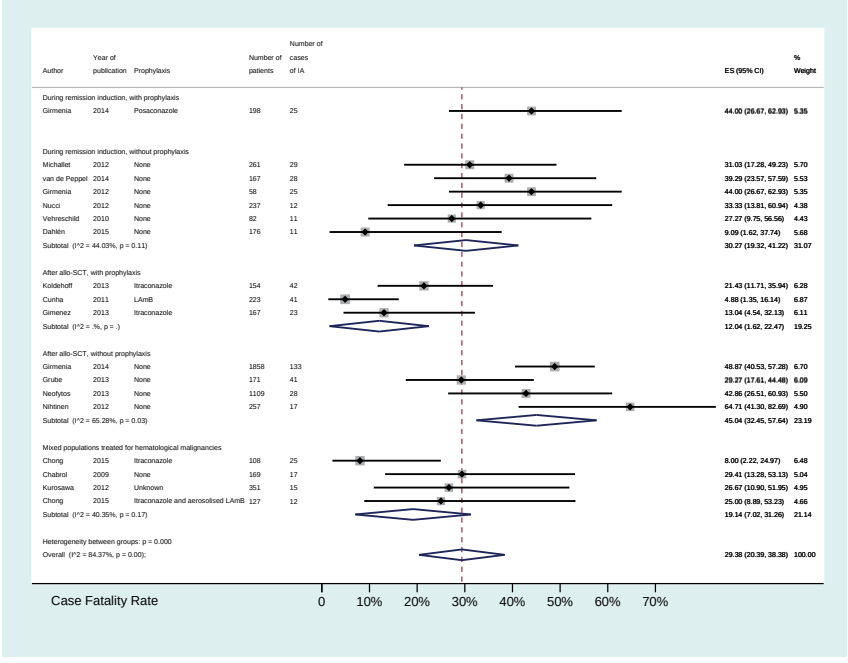
Study population	Notes	# pts	# IA cases	IA incidence	CFR within 100 days	CFR provided by author	Lost to follow-up
During RI for different HM and after allo-HSCT		203	18	8.9%	NA	NA	<5%
During RI-therapy for AML or ALL	Renovation work during study period	169	17	12.4%	29%	NA	None
After allo- or auto-HSCT		104	6	5.8%	NA	NA	None
During RI-therapy with azacitidine as salvage therapy		64	6	9.3%	33%	NA	NA
During RI-therapy for AML and ALL	Renovation work during study period	91	9	9.9%	NA	NA	NA
Different HM treated with Alemtuzumab as frontline, salvage or conditioning regimen.		182	15	8.2%	NA	NA	NA
After allo or auto-HSCT		429	17	4.0%	NA	NA	NA
During RI or after allo-HSCT for AML		1059	60	5.7%	NA	NA	NA
After allo-HSCT from unrelated donor		135	32	23.7%	NA	NA	NA
AML or high-risk MDS treated with Azacitidine		121	1	0.8%	100%	NA	NA
During RI for different HM and after allo- HSCT		53	9	16.9%	11%	NA	NA
During RI for different HM and after allo- HSCT		129	14	10.9%	NA	NA	NA
		2739	**204**	**7,4%**	**27% (in 33 cases of IA)**		
After allo- or auto-HSCT		45	2	4.4%	50%	Yes	NA
During RI for different HM and after allo- or auto-HSCT		140	18	12.9%	NA	NA	3.8%
After allo-HSCT	Questionnaire-based	351	15	4.2%	27%	Yes	NA
During RI-therapy for different HM	Questionnaire-based	2224	8	0.4%	0%	Yes	NA
After allo-HSCT		96	0	0%	NA	NA	NA
During RI-therapy		82	3	3.7%	0%	NA	None
During RI-therapy for AML	Renovation work during study period	146	8	5.5%	50%	Yes	6.2%
During RI-therapy for ALL	Renovation work during study period	49	0	0	NA	NA	6.2%

Table 1. Characteristics of all included studies (continued)

Author	Study design	Country	Pub year	Study period	Prophylaxis
Loschi et al(75)	Cohort	France	2015	2003-2008	Unknown
Loschi et al(75)	Cohort	France	2015	2003-2008	Unknown
Crude total					
Crude total among all populations with prophylaxis					
Crude total among all populations without prophylaxis					
Crude total among all patient categories (including mixed or unknown prophylaxis)					

Legend: Pub year denotes year of publication, CFR: case fatality rate, IA: invasive aspergillosis, AML: acute myeloid leukaemia, MDS: myelodysplastic syndrome, ALL: acute lymphoid leukaemia, RI: remission-induction, Allo-: allogeneic, Auto-: autologous, HSCT: haematopoietic stem cell transplantation, LAmB: liposomal amphotericin B, AmbD: amphotericin B deoxycholate, HM: haematological malignancies, pts: patients, NA: not available

Figure 3. Meta-analysis of case fatality rates within 100 days of diagnosis of invasive aspergillosis in different subpopulations



Legend: Allo-SCT denotes allogeneic stem cell transplantation; ES estimate; CI confidence interval. The black dot represents the individual studies effect. The size of the grey squares represents the study weight according to the random effects model. The black lines represent the 95% confidence intervals of different studies. The diamonds represent the overall (or subgroup) effects, the outer edges of the diamonds represent the 95% confidence intervals.

Study population	Notes	# pts	# IA cases	IA incidence	CFR within 100 days	CFR provided by author	Lost to follow-up
After allo-HSCT	Renovation work during study period	58	1	1.7%	0%	Yes	6.2%
After auto-HSCT	Renovation work during study period	249	4	1.6%	0%	Yes	6.2%
		3440	**59**	**1,7%**	**22% (in 41 cases of IA)**		
		# pts	**# IA cases**	**IA incidence**	**CFR within 100 days**		
		4896	**372**	**7,6%**	**23% (in 94 cases of IA)**		
		8479	**625**	**7,4%**	**39% (in 410 cases of IA)**		
		16815	**1056**	**6.3%**	**33% (in 645 cases of IA)**		

DISCUSSION

Summary

This meta-analysis summarises all relevant published findings related to incidence and CFR of IA in cohorts of patients treated for haematological cancer in the age after the 2008 revised EORTC criteria for the diagnosis of IA (9). Incidence rates varied between 4% and 12% depending on the treatment phase and use of prophylaxis. The incidence of IA is lower in populations with mould-active prophylaxis, which is most evident in the pre-HSCT population. However, the incidence remained substantial despite prophylaxis with 4% in the pre-HSCT, and 9% in the post-HSCT population.

Efficacy of antifungal prophylaxis

No conclusions can be drawn concerning the efficacy of the type of antifungal prophylaxis due to the study heterogeneity and non-comparative nature of the included studies. Most populations in the pre-HSCT period used posaconazole as antifungal chemoprophylaxis. In the post-HSCT period more different types of prophylaxis were used, possibly contributing to the observed increased heterogeneity in this group.

Case fatality rates

The pooled CFR within 100 days was 29% with a large variety between studies. The relatively large variety is possibly due to the low number of participants and population heterogeneity. The CFR did not evidently differ between treatment phases or between populations with and without use of antifungal prophylaxis. As a higher fungal load is associated with more apparent radiological signs, increased chance of successful culture and higher levels of galactomannan in serum or BAL-fluid, increased diagnostic certainty impacts CFR as well. Only 6 of our

included studies had available information on the CFR of proven cases of IA aggregating to a CFR of 72%, which is remarkably higher than the total CFR.

The alleged association between breakthrough infection (i.e. occurrence of IA despite adequate mould-active prophylaxis) and increased mortality that was earlier reported (22, 47, 48) was not found in our meta-analysis. Our data contradict the hypothesis that occurrence of infection despite adequate triazole-based prophylaxis is more often caused by triazole-resistant *Aspergilllus* (22, 47). As resistant infection is associated with increased mortality, it would be expected to impact the case fatality rates (7, 49).

Recently, an increasing number of studies describing mortality after IA report the cause of death or the probability of IA-related death (50). From a clinical point of view, and supported by literature (50-52), death attributable to IA is hard to establish in the haematological patient that usually faces multiple competing risks with high mortality during their treatment. Factors associated with IA can contribute to an increased mortality risk, independently of the presence of IA (29, 41, 53). As an alternative to presenting IA-related death, a relatively short CFR of 100 days after diagnosis has been used. It is however plausible that a considerate proportion of patients die from a cause that has no relation to IA. Therefore, the crude mortality rates overestimate the IA-attributable mortality, although this is difficult to quantify (54, 55).

Results in context of existing evidence

To this date, no systematic studies on the incidence of IA in the era after the 2008 revised EORTC criteria have been published in English literature. CFRs in this population have last been presented in a meta-analysis published in 2001 which reports a CFR of 49.3% for patients with leukaemia or lymphoma and 86.7% for patients that underwent a HSCT (2). These numbers contrast with the aggregate rate of 29% in our meta-analysis. Since the publication of the aforementioned paper, the introduction of novel antifungals and improvements in diagnostic techniques have been important factors in diminishing the CFR. Our results are consistent with more recent studies of large cohorts of patients with haematological malignancies. Published in the pre-2008 revised EORTC-definitions era, the SEIFEM-cohort published by Pagano et al (1) in 2006, yields an overall incidence of invasive mould infection of 7% in the pre-HSCT period in AML patients and an overall CFR within 30 days of 38%.

Because this meta-analysis only included studies from the period after implementation of the EORTC-definitions, it is important to take the effect of the guidelines themselves on the reporting of incidence and mortality in consideration. Studies that have retrospectively reclassified patients at risk for IA found that implementation of the new criteria decreases the incidence of probable or proven IA (10, 11). As an increased diagnostic certainty is associated with an increased CFR (10), it is to be expected that the CFR would be higher after implementation of the new criteria. This phenomenon is not observed when comparing our estimate to the aforementioned literature.

Strengths and weaknesses

An important strength of this study is the large quantity of data that could be analysed. A total of 49 studies describing 16815 patients were included in the analysis, of which 7915 remained in the subgroup analyses. Additionally, study authors have been contacted concerning incomplete fatality data to provide a more complete overview. Another strength is that all presented CFRs originate from studies where incidence rates were also available. This allows for interpretation of the data in specific populations at risk of IA. Weaknesses of the study mainly comprise different sources of bias. Selection bias is of general concern in all observational studies and comparisons that we can make between groups are in a non-randomized setting. Also, study heterogeneity contributes to increased difficulty of the interpretation of differences between groups. Although both the treatment of haematological malignancy and diagnosis of IA are globally regulated in guidelines, small individual differences between study centres are expected to impact the comparability of the different studies. To account for this observed heterogeneity, a random effect model has been used; however, this does not remove it. Publication bias is a known problem of systematic reviews and could have influenced our conclusions. However, since both an unexpectedly high or low incidence of IA could improve the chances of publication, this possible source of bias is expected to have a minor effect as compared to meta-analyses measuring treatment effects. This was confirmed by the lack of evident asymmetry in the forest plots sorted by number of study participants.

Conclusion

Our first conclusion is that incidence rates are substantial despite the implementation of universal antifungal prophylaxis. Secondly, the pooled CFR of IA amounts to 29%, a relatively low rate when compared to historical cohorts and the last published meta-analysis (2). This study summarizes data of global occurrence and mortality of IA in a comprehensive manner and provides the background necessary for the rationale of preventive measures. It is shown that IA has an important clinical impact in patients treated for haematological malignancy. The disease poses a relevant threat in the treatment of haematological cancer worldwide. To attempt to reduce the burden, new solutions in the field are necessary as antifungal drugs are shown to be imperfect in both treatment and prevention of IA.

Therapeutic or prophylactic failure of antifungal agents, both associated with inherently limited drug efficacy and rising resistance all over the world, are currently the greatest challenges that we face in the field. Tackling the problem of resistance and managing breakthrough infection becomes an increasingly important part in the management of IA and we currently have only limited possibilities to do so. Future research should aim to provide clinicians with better options in facing these challenges.

These outcomes inform scientists and other stakeholders with evidence about the current burden of IA. This information may be globally used to direct, implement and improve antifungal stewardship programs.

Contributions: RP performed the data collection and wrote the first draft of the manuscript. RP and MB extracted the data in mutual agreement. RP, MB and LV were involved in the design of the study. Analyses were performed by RP in collaboration with OD. All authors critically revised all drafts of the manuscripts and approved the final version.

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Chapter 3

Relapsed and secondary disease drive the risk profile for invasive aspergillosis prior to stem cell transplantation in patients with acute myeloid leukaemia or myelodysplastic syndrome

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ABSTRACT

Introduction: Due to both the disease and intensive chemotherapy, patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) are at risk for invasive aspergillosis (IA) even prior to stem cell transplantation (SCT). In times of increasing triazole resistance and changing use of antifungal prophylaxis, insight in risk factors is needed to improve strategies to prevent IA in this population.

Methods: Consecutive patients who received ≥1 course of remission-induction therapy for AML or MDS from 2005 to 2012 at the Leiden Academic Medical Centre were included. No standard antifungal prophylaxis, but an assertive diagnostic protocol for diagnosis of suspected fungal infection was in place. IA was classified according to revised EORTC criteria. Potential predisposing clinical characteristics for IA were analyzed by uni- and multivariate analyses.

Results: In 45 (25%) of 184 included episodes (167 patients), IA was diagnosed prior to SCT (5% proven, 59% probable and 37% possible IA). A multivariate Cox regression model demonstrated that relapsed AML (HR 2.4, 95%CI 1.1-5.1, p=0.02), secondary AML (HR 5.2, 95%CI 2.3–11.8, p<0.001) and prolonged duration of neutropenia, i.e. >60 days of neutropenia (HR 2.2, 95%CI 1.2–4.0, p=0.01) were independent risk factors for IA. Use of G-CSF showed a trend toward protection against the occurrence of IA (HR 0.37, 95%CI 0.13–1.0, p=0.06).

Conclusions: Relapsed AML, secondary AML and duration of neutropenia were independent factors determining the risk signature for development of IA prior to SCT. The results provide further guidance for antifungal stewardship programs when integrating individual patient 'tailored' decision making in antifungal prophylaxis strategies.

INTRODUCTION

Invasive aspergillosis (IA) is a relatively common infectious complication in patients with haematological malignancies (1). Particularly patients diagnosed with acute myeloid leukaemia (AML) or high-risk myelodysplastic syndrome (MDS) are prone to development of IA due to both the nature of the disease as well as the treatment they receive (2, 3). The numbers of functional neutrophilic granulocytes, which are considered most important in host defence against invasive fungal infection, are severely decreased in many patients with AML or MDS (4). In addition, long periods of complete granulocytopenia occur as a result of treatment with intensive chemotherapy needed to achieve remission of disease. Previous clinical studies that assessed risk factors for IA particularly focused on the time period after the procedure of stem cell transplantation (SCT) and on populations with a wider range of haematologic conditions than only AML and MDS. In these studies, delayed neutrophil engraftment, chronic graft versus host disease (cGVHD), secondary neutropenia, relapse after transplantation, use of corticosteroids and older age were found to be associated with the occurrence of IA after SCT (5-7). However, specific studies that assessed the clinical characteristics, and their magnitude of effect, on the risk of development of IA in the pre-transplantation period are scarce or outdated (8).

At present, the anticipated incidence rates of IA in the post SCT period have been reduced by implementation of chemoprophylaxis policy guidelines issued by internationally accepted expert committees (9-11). However, these and other guidelines consider the evidence for the efficacy of prophylaxis in the period prior to SCT to be incomplete. Some transplant centres have developed their own protocols for antifungal prophylaxis, based upon local incidence of IA and observed rates of emerging resistance of *Aspergillus* isolates (12-14). Due to limited scientific data and consensus, practices for antifungal prophylaxis in patients with AML or high risk MDS prior to SCT vary widely (15, 16).

Nevertheless, a more detailed understanding of the clinical characteristics that define the risk for later development of IA is imperative in individual risk-benefit assessment for antifungal prophylaxis in this setting. Furthermore, this knowledge would contribute to our ability to develop and study chemo-prophylactic strategies in this population. In this study we assessed potential risk factors for the occurrence of IA prior to SCT in a cohort of patients with AML or high risk MDS in the pre-transplantation period.

METHODS

Setting and study population

The study was performed at the Leiden University Medical Center, a tertiary care and teaching hospital in the Netherlands committed to extensive solid organ and haematological transplantation programs. The study cohort consisted of all patients admitted between January

1st 2005 to January 1st 2012 for treatment of AML or high risk MDS and who received at least one course of remission-induction chemotherapy. In our institution, most patients with AML or high risk MDS achieving complete remission with intensive chemotherapy proceed to SCT. Patients were identified and included through the admission database of the department of haematology. This database was cross-linked with the hospital's pharmacy database containing all patients that received either voriconazole (Vfend®) or liposomal amphotericin B (Ambisome®) for the treatment of IA. These were the only antifungal drugs allowed for this indication. Patients that met the revised 2010 EORTC criteria (17) of possible, probable or proven IA were regarded as patients with confirmed IA. Potential IA cases that did not meet these criteria were excluded from the analyses. Treatment of AML and MDS as well as the diagnostic protocol and treatment of IA had not been subject to major modifications during the complete period of study. In case of suspected fungal infection, i.e. in patients with persistent fever for >3 days without other evident cause, a CT-scan of the lungs was performed. According to protocol, intrapulmonary abnormalities were sampled by bronchoalveolar lavage (BAL) or lung biopsy. Direct staining for fungi as well as fungal cultures and galactomannan assay were performed on obtained BAL-fluid samples. The galactomannan assay (or a beta-D-glucan assay) was not used for routine screening for invasive fungal infections. No standard primary chemoprophylaxis for the prevention of infection with filamentous fungi was used. The study was endorsed by the hospital's ethical committee.

Collection of data and definitions

Data about age, sex, use of glucocorticoid drugs and characteristics of the haematological disease were retrieved from the hospital's electronic patient file system. Data about pre-existing co-morbidities (lung disease, diabetes or previous malignancies) were also extracted from the system. The studied variables with regard to underlying haematological disorder included disease specific genetic abnormalities, risk group of disease according to the WHO classification system, treatment with granulocyte-colony stimulating factor (G-CSF), chemotherapeutic regimen and the number of induction therapies needed to attain remission of disease. Data concerning a potential diagnosis of IA (i.e. CT-scan report, results of BAL-fluid analysis and serum galactomannan test results) and diagnostic classification of IA according to the 2010 revised EORTC criteria (17) were documented. The date of diagnosis of IA was defined as the day of start of antifungal treatment. The duration and frequency of neutropenic periods were obtained by using the history of available white blood cell counts for each patient. Neutropenia was defined as $<500 \times 10^6$ neutrophilic granulocytes/L. For patients with IA, the length of the neutropenic period was determined as the number of days with neutropenia before the diagnosis of IA. For the purpose of this study, the follow-up period for all patients spanned from the day of diagnosis of the haematological disease to either the day of SCT or death.

Statistical analysis

Univariate comparison of the association between potential risk factors and IA was performed by Cox regression model. Results are reported as hazard ratio's (HR) with 95% confidence intervals (CI95%). The variables included in the multivariate Cox proportional hazards model were selected based on either $p<0.10$ in the univariate regression analysis and/or plausibility. Statistical analysis comparing the data of patients with and without the diagnosis of IA was performed with SPSS 20.0 statistical software.

RESULTS

Study cohort characteristics

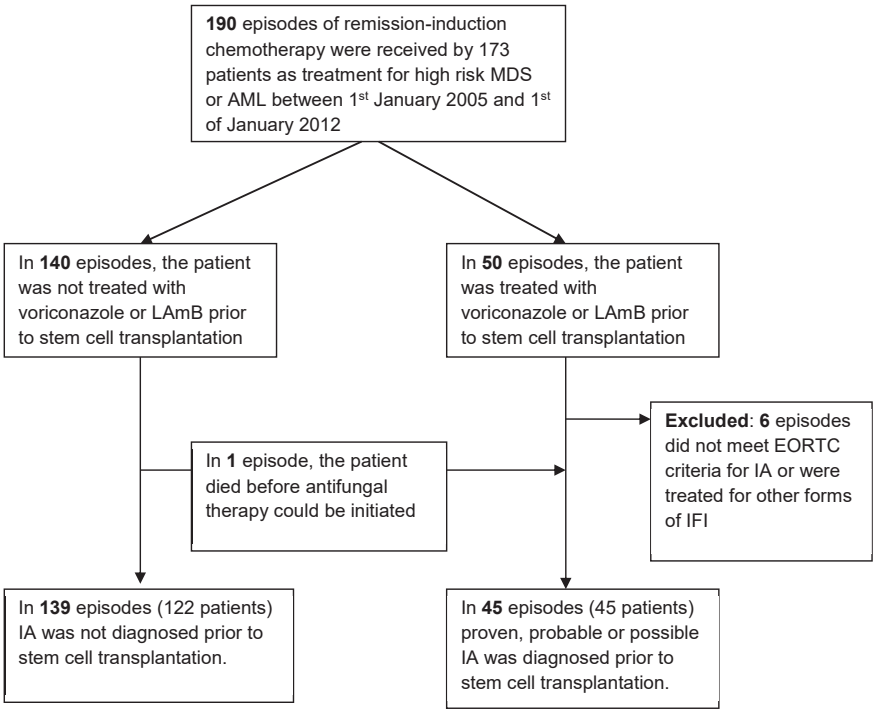
The total cohort of patients receiving remission-induction therapy for AML or high-risk MDS consisted of 173 patients. Of these patients, 87 were male and median age was 54 years. Relapse of haematological disease occurred in 27 patients, of which 17 were treated in our hospital during the first as well as the second episode of their disease, adding an extra episode to the total. The other 10 patients with relapsed disease were first treated in another hospital and only contribute their second episode to the study cohort. None of these patients were diagnosed with IA during their first treatment episode. Thus, a total of 190 episodes of remission-induction therapy were registered between 2005 and 2012.

The database of the department of pharmacology showed that, prior to SCT, treatment with voriconazole and/or liposomal amphotericin B was prescribed during 68 (36%) of these episodes. Reasons for prescription of fungicidal drugs for other reasons than treatment of IA were: chemoprophylaxis for IA (n=1), aborted empirical treatment (n=4) and fungal infections other than IA (n=1). Of the remaining 62 episodes with potential IA, 18 were added to the group of patients without IA because IA occurred and was treated after the event of SCT. One patient died with probable IA before therapy could be administered and was added to the group of patients with IA. This resulted in a total number of 184 episodes (45 in which IA was diagnosed, and 139 without diagnosis of IA) included in the analyses (figure 1).

SCT was performed after 100 (54%) episodes of remission-induction chemotherapy. In a total of 71 episodes (39%) the patient died prior to SCT. Of these 71 patients, 23 (32%) suffered from IA in the period between start of remission-induction therapy and their death. For the remaining 13 episodes (7%), patients were not transplanted and still alive at the end of the period of study. The uncorrected mortality risk before transplantation was higher in episodes with IA as compared to episodes without IA (51% vs. 35% of patients, respectively, HR 1.7, 95%CI 1.0 - 2.8, p=0.047).

The incidence of IA did not increase over time during the period of the study. In 9 (20%) cases in which *Aspergillus* was cultured from either sputum or BAL samples, a common distribution of *Aspergillus* spp. was found: eight A. fumigatus and one A. flavus. Only one A. fumigatus was found to be resistant for itraconazole, posaconazole and voriconazole.

Figure 1. Study cohort



Legend: MDS: myelodysplastic syndrome; AML: acute myeloid leukaemia; IA: invasive aspergillosis; LAmB: liposomal amphotericin B.

Risk factor analysis

The results of the univariate risk factor analysis for the occurrence of IA are shown in table 1. Relapsed AML (HR 2.2, 95%CI 1.1-4.3) or secondary AML (HR 3.8 95%CI 1.7-8.0) and duration of neutropenia over 60 days (HR 2.2 95%CI 1.2-4.0) enhanced the risk for developing IA. Any form of pre-existent lung disease (HR 1.8, 95%CI 0.9-3.6) showed a trend toward an increased risk. Treatment with G-CSF in the time phase before transplantation was associated with a decreased risk (HR 0.4 95%CI 0.1-1.0) for the occurrence of IA.

The results of the multivariate analysis, performed using a Cox proportional hazards model, are shown in Table 2. A cumulative number of more than 60 days of neutropenia (HR 2.2, 95%CI 1.2-4.0, p=0.01), secondary AML (HR 5.2, 95%CI 2.3-11.9, p<0.001) or relapsed AML (HR: 2.4, 95%CI 1.1- 5.1, p=0.02) were independently associated with the occurrence of IA (table 3). Treatment with G-CSF remained to show a trend towards a protective effect (HR 0.37, CI95% 0.13-1.0, p=0.06). The addition of age and sex to the model did not affect the calculated estimates.

Table 1. Univariate analysis of clinical characteristics of patients receiving chemotherapy for treatment of AML or high risk MDS with and without invasive aspergillosis

Variable	Episodes with IA No. (%)	Episodes without IA No. (%)	Hazard ratio (95%CI)	p-value
Number of episodes (total 184)	45	139		
Number of patients (total 167)	45	122		
Age (years), median (IQR)	54 (46-64)	54 (47-64)	1.0 (0.98-1.03)	0.87
Age >60	16 (36)	54 (39)	0.9 (0.5-1.6)	0.87
Sex (male)	22 (49)	72 (52)	0.9 (0.5-1.6)	0.70
Disease characteristics				
Secondary AML	8 (17)	4 (3)	3.8 (1.7-8.0)	0.001
Relapsed AML	11 (25)	17 (12)	2.2 (1.1-4.3)	0.04
Poor or Very poor prognosis[#]	24 (53)	68 (49)	1.2 (0.7-2.2)	0.49
>1 Remission-Induction-course	13 (29)	28 (20)	1.4 (0.8-2.4)	0.22
Previous lung disease (total)^	10 (22)	18 (7)	1.8 (0.9-3.6)	0.10
Previous pneumonia	6 (13)	11 (8)	1.5 (0.6-3.5)	0.37
COPD	3 (7)	5 (3)	2.4 (0.7-7.7)	0.15
Asthma	2 (4)	1 (1)	2.8 (0.7-11.6)	0.15
Lung embolism	0	2 (1)	NA	NA
Lung cancer or metastases	1 (2)	0	NA	NA
Diabetes	4 (8)	10 (20)	1.2 (0.4-3.4)	0.70
Medication use				
G-CSF	4 (8)	32 (23)	0.4 (0.1-1.0)	0.05
Corticosteroids	14 (31)	55 (38)	0.8 (0.4-1.5)	0.46
Duration of neutropenia (days),median (IQR)	65 (32-90)	47 (26-60)	1.007(1.001-1.014)	0.02
Neutropenia at diagnosis	14 (33)	31 (22)	1.6 (0.9-3.0)	0.14
> 30 days of neutropenia	35 (77)	91 (65)	1.4 (0.7-3.0)	0.29
> 60 days of neutropenia	23 (51)	35 (25)	2.2 (1.2-4.0)	0.008

Legend: IA denotes invasive aspergillosis; 95%CI: 95% confidence interval; IQR: interquartile range; G-CSF: granulocyte-colony stimulating factor; AML: acute myeloid leukemia. Values represent numbers and percentages unless indicated otherwise; ^: Lung disease defined as any lung disease stated in the patient's medical history, #: Risk classification according to national HOVON-102 protocol; $: Neutropenia defined as a blood granulocyte count <500 x10^6neutrophilic granulocytes/L.

Table 2. Multivariate analysis: Cox proportional hazards model of clinical characteristics of patients receiving chemotherapy for treatment of AML or high risk MDS with and without invasive aspergillosis

Variable	Hazard ratio (95%CI)	p-value
Previous lung disease^	1.7 (0.8-3.6)	0.16
G-CSF used	0.4 (0.1-1.0)	0.06
Secondary AML	5.2 (2.3-11.8)	<0.001
Relapsed AML	2.4 (1.1-5.1)	0.02
Neutropenia[#] at presentation	1.5 (0.8-2.9)	0.19
>60 days of neutropenia	2.2 (1.2-4.0)	0.01

Legend: G-CSF: granulocyte-colony stimulating factor; AML: acute myeloid leukaemia ^: Previous lung disease defined as any lung disease stated in patient history, e.g. COPD or previous pneumonia; #: Neutropenia defined as a blood granulocyte count <500 $x10^6$ neutrophilic granulocytes/L;

DISCUSSION

Although antifungal prophylaxis has been proven effective in the prevention of IA during chemotherapeutic treatment for AML and MDS in the phase prior to SCT (2, 18), national and international guidelines do not explicitly recommend primary prophylaxis to prevent IA in this setting. The Dutch national 2008 SWAB (Dutch Working Party on Antibiotic Policy) guideline on fungal infections advises to consider primary prophylaxis for this population if the local incidence of infection with filamentous fungi is high (9). The IDSA (Infectious Disease Society of America) 2008 guideline recommends primary antifungal prophylaxis in neutropenic patients with AML or MDS who are at high risk for IA, but specifically notes that further study is needed to determine which subpopulations benefit most from this approach (10). In addition, the European ECIL (European Conference on Infections in Leukaemia) 2009 updated guideline provides rationale for either a mould directed diagnostic approach in combination with fluconazole (for the prevention of invasive candidiasis) as well as for the use of primary prophylaxis aimed at the prevention of both infection with *Candida* spp. and filamentous fungi (11). In medical centres worldwide performing SCT, the incidence of IA and choice of antifungal prophylactic strategies vary considerably. In agreement with antibiotic stewardship principles in maintaining an overall restrictive antibiotic policy to inhibit emerging resistance and to limit unnecessary drug toxicity, our centre had opted for an assertive mould directed diagnostic approach and subsequent empirical treatment of IA. At that time it was largely unknown that resistance against triazole drugs was emerging from the environment, rather than through selective pressure by the use of these agents in the clinical setting.

In this study, we found an average incidence of (probable and proven) IA in 15% of episodes, i.e. during chemotherapy prior to SCT, in patients with AML or MDS. Prior to our study, the incidence of IA in this population was not systematically monitored in our institution. The incidence of IA did not increase during the period of study (data not shown) and an expected

distribution of possible, probable and proven IA was found (3, 19). Directly after this finding, the use of primary antifungal prophylaxis was implemented in our treatment protocols for this patient population according to the national guidelines. The fact that chemotherapeutic treatment protocols for MDS and AML as well as applied diagnostics for IA were not altered during the period of study enabled us to investigate whether specific clinical patient characteristics would outline individual patients and subpopulations at increased risk for IA prior to SCT.

The multivariate Cox-model, expressing the adjusted risk as hazard ratio (HR), showed the strongest independent correlations between treatment for secondary or relapsed AML and the occurrence of IA. This increased risk may be caused by chemotherapeutic treatment these patients received before they started intensive chemotherapy for treatment of their current AML or MDS. But other factors e.g. poorer nutritional status may additionally influence the risk state with regard to IA in this subpopulation. Furthermore, a granulocyte count less than 500 $x10^6$ neutrophilic granulocytes/L at the time of diagnosis or prolonged period of neutropenia (i.e. >60 days) increased the risk for development of IA. Notably, age, sex, type of treatment and the cytogenetics that determine the prognostic classification of the haematological malignancy were not associated with the occurrence of IA. Treatment with G-CSF showed a trend toward a reduced risk of IA.

Since the overall risk (24%) of receiving treatment for IA in the period pre-SCT was high, the study outcome may not suggest benefit of the implementation of a selective prophylactic policy based on the risk factors found, at first instance. For example, prescription of primary antifungal prophylaxis to a high risk group consisting of patients that either suffer from secondary or relapsed disease or a period of neutropenia longer than 60 days, results in an estimated number needed to treat (NNT) to prevent 1 case of 2.5 while at a maximum preventing 76% of all cases. Although this NNT is very low, prophylaxis for the entire cohort conveys a NNT of 4.1, with a maximised prevention of IA for all cases.

However, implications reach further as the use of primary antifungal prophylaxis over a prolonged period of time may be complicated by several factors. First, considerable toxicity of the antifungal agents has been reported (20, 21). Prophylaxis with voriconazole is associated with a substantial frequency of severe adverse events, e.g. in one recent study 13% of patients developed liver toxicity and in 5% eye dysfunction was observed (22). Moreover, recent studies show an increasing incidence of resistance of *Aspergillus* spp. against triazoles (14, 23). Although in our cohort only one azole-resistant *Aspergillus* was cultured, a positive culture from BAL or sputum was available in only 9 (11%) cases. In the near future, resistance will increasingly complicate prevention by prescription of primary prophylaxis using triazoles. This may result in preference of a monitoring policy for this group of patients, or selective prophylaxis with more toxic agents for patients at high risk. Knowledge of predictive factors like those identified from our data may provide guidance in balanced decision-making with regard to a more individualized approach toward prescription of primary prophylaxis.

Some limitations exist due to the retrospective design of the study and the size of the cohort. The results may not be applicable to other populations of patients at risk for IA, e.g. for patients undergoing treatment for ALL. Because the study population at risk for IA consisted mainly of patients with AML rather than MDS, subanalyses of risk variables for patients with MDS could not be made. However, subanalyses of patients with AML only did not affect the outcome. Furthermore, the composed set of potential risk factors that was studied may not be complete. Strengths of the study include that treatment for AML or MDS as well as the diagnostic protocol for IA were unchanged during the entire period of study and that detailed clinical data was available for all patients. Furthermore, the availability of exact data about the neutropenic periods of all patients in the cohort provides a reliable insight in the value of the granulocyte count in predicting the occurrence of IA.

Within antifungal stewardship programs, the outcomes provide a basis for the design of a tailor-made prophylactic approach, which balances the prevention of a prevalent disease with toxicity of therapy and increasing azole-resistance of *Aspergillus* spp. Future clinical trials should also assess the costs and benefits of risk factor-based prophylactic strategies for the prevention of IA. Importantly, the results may already be applied to clinical practice since decisions about discontinuation of antifungal prophylaxis in individual patients experiencing side effects of triazole agents should always be balanced against the assessment of the patient's risk for development of IA.

Potential conflicts of interest. All authors: no conflicts

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Chapter 4

A new time-dependent approach for assessment of the impact of invasive aspergillosis on short- and long-term survival of patients with haematological malignancy.

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ABSTRACT

Introduction Invasive aspergillosis (IA) has been reported to yield high mortality rates. Patients with an unfavourable prognostic haematological disease not only have a higher probability to develop IA but are also more likely to die due to causes directly related to the underlying disease. This complexity of risk mechanisms confounds the causal interpretation of IA occurrence and mortality. Full consideration of the changing patient characteristics over time is necessary to obtain reliable estimates of the correlation between IA and mortality.

Methods We studied the effect of IA on mortality in 167 consecutive patients starting with remission-induction therapy for AML or MDS. Most of these patients continued to haematopoietic stem cell transplantation (HSCT). No standard antifungal prophylaxis was administered in the period before HSCT. Survival analyses were performed to determine risk estimates of IA for different phases of treatment before and after HSCT. Time-dependent adjustment for confounding variables was performed using Cox proportional hazards models.

Results IA was diagnosed in 55 of 167 enrolled patients. Before HSCT, adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) on mortality after the diagnosis of IA were 3.5 (1.7-7.5), 2.0 (0.69-5.9), 2.3 (0.79-6.8) and 0.80 (0.49-1.4) within 30 days, between 30 and 60 days, between 60 and 90 days or more than 90 days respectively. A similar pattern was observed after HSCT. The occurrence of IA did not significantly influence the decision to follow through with HSCT.

Discussion The results provide new insights in short- and long-term survival of patients diagnosed with IA. A significantly increased mortality risk was only observed in the first month after diagnosis of IA. No unfavourable association with mortality was observed in the later course of treatment. The occurrence of IA did not affect the probability of attaining HSCT in our population.

INTRODUCTION

The prognosis of patients with haematological malignancies has improved over the past decades by the development of new anti-leukemic agents, intensification of chemotherapeutic regimens and the use of haematopoietic stem cell transplantation (HSCT) (1, 2). However, these therapeutic advances have increased the risk of invasive fungal infections, especially in patients undergoing intensive chemotherapy or haematopoietic stem cell transplantation (HSCT) (3, 4). Patients treated for acute myeloid leukaemia (AML) or high-risk myelodysplastic syndrome (MDS) are particularly prone to the development of IA due to the impact of both the intensive chemotherapy and the effect of the disease itself on the granulocyte function, which is considered the most important host defence against invasive fungal infection (5, 6).

Despite currently available antifungal prophylaxis and treatment, development of IA still leads to high mortality rates (3, 4). The most important risk factors for IA in patients with haematological malignancies have been described in several studies, comprising both different host factors (e.g. prolonged periods of neutropenia) and different treatment characteristics (7-9). In AML and MDS many of the factors that convey a higher risk for IA also independently contribute to treatment failure, likelihood of other complications and mortality due to a combination of high-risk treatment protocols and severity of the underlying disease. Additionally, because pre- and post-transplantation states are very distinct in terms of host immunity, many risk factors associated with mortality have been found to differ before and after transplantation (10-12). This complexity of risk mechanisms confounds the causal interpretation of the association between occurrence of IA and resulting mortality. Because of these complex mechanisms, the correlation between the occurrence of IA and its effect on long-term survival (i.e. longer than 6 weeks) in patients with AML or MDS remains to be clarified. In-depth analyses of patient characteristics, treatment phase and competing risks over time are needed to obtain reliable estimates of the short- and long-term impact of IA on mortality in this vulnerable patient population.

In this study, we aimed to present a new and comprehensive method to study the short and long term effect of IA on the survival of patients receiving treatment for AML or high-risk MDS.

METHODS

Setting and study population

The study was performed at the Leiden University Medical Center, a tertiary care and teaching hospital in the Netherlands, which is committed to solid organ and haematological transplantation programs. All patients treated for AML or high-risk MDS between January 1st 2005 and December 31st 2011 with at least one course of remission-induction chemotherapy were included in our study. Follow-up started at diagnosis of AML or MDS and ended on July 1st 2012 or

with the death of the patient. Almost all patients proceeded to HSCT after achieving complete remission with intensive chemotherapy. There were no considerable changes in treatment of AML or high-risk MDS, diagnostic strategies or antibacterial prophylaxis and empirical therapy strategies within the time period of the study. During the study, patients did not receive systemic mould-active antifungal prophylaxis. Periodic screening for invasive fungal infection by serum galactomannan or beta-D-glucan assays was not performed. A standardised protocol for diagnosis of suspected fungal infection was in place. In case of suspected fungal infection, e.g. in patients with persistent fever for at least 3 days without another evident cause, a HRCT-scan of the lungs was performed. If the scan showed intrapulmonary abnormalities, bronchoalveolar lavage (BAL) and/or lung biopsy was performed. BAL-fluid samples were subjected to direct staining for fungi, fungal culture and galactomannan assay. In addition to the BAL, or in case a BAL was not possible, serum galactomannan tests were executed in neutropenic patients suspected for IA. A galactomannan of > 0.5 in fluid obtained by BAL or >1.0 in serum was considered a positive test result. After review by the local ethics committee, a waiver for the need of informed consent was issued.

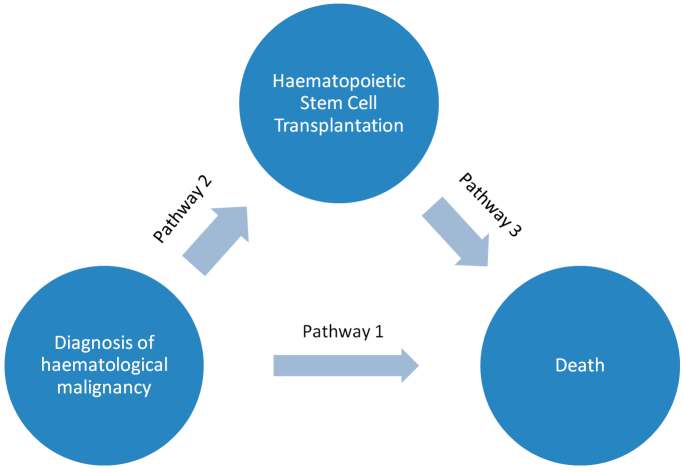
Collection of data and definitions

Patient data were extracted from the electronic patient file system. These included age, sex, pre-existing co-morbidities (lung disease, diabetes or previous malignancies) and characteristics of haematological disease. Collected characteristics were disease-specific genetic abnormalities, risk group of disease according to the WHO classification system, use of granulocyte-colony stimulating factor (G-CSF) in any treatment phase, the chemotherapeutic regimen and the total number of induction therapies needed to achieve remission of the haematological disease. The database was anonymised on completion and the decryption key was stored on a secure hospital drive. A single patient could contribute two disease episodes to the cohort if a relapse occurred within the study period. A new episode was counted as soon as the diagnosis of relapsed disease was confirmed and started with a new pre-transplant period. Specific diagnostic tests concerning the diagnosis of IA as described in the 2008 revised EORTC criteria were collected (i.e. CT-scan, BAL-fluid analysis and serum galactomannan tests). Subsequently, each case was categorised accordingly as possible, probable, proven or indefinite IA. Patients meeting the revised 2008 EORTC criteria (13) of having possible, probable or proven IA were regarded to have IA. Patients that were treated for fungal infection other than IA or were empirically treated with antifungal agents but did not meet the diagnostic criteria for at least a possible IA were censored from the moment antifungal therapy was initiated. As these patients are in a state in which the chance of occurrence of at least possible IA is significantly decreased, they could not be considered a representative control. The date of IA diagnosis was defined as the day of start of antifungal treatment. The laboratory results for every patient were examined for appraisal of neutropenic status. A neutropenic period was defined as the time between the first day of neutropenia (i.e. a neutrophil count below 500 cells/µl) and the first day of resolution of neutropenia (neutrophil counts rising above this number).

Statistical analyses

The time periods before and after transplantation are two very distinct phases with regard to both treatment and immunological host factors. Consequently, many of the studied variables can act differently from one phase to the other. Because reaching HSCT is an obligatory step in achieving definite remission and therefore is a critical factor in the survival of these patients, it was treated as an intermediate endpoint. We aimed to divide the follow-up data accordingly and therefore the analysis contains three separate pathways within the disease episode, which are visualised in a multi-state model (14): firstly, a route to death in the pre-transplant period; secondly, a route to transplantation; thirdly, a route to death in the post-transplant period (figure 1). Correspondingly, a set of three survival analyses based on Cox regression models accounting for the different phases in treatment were constructed, considering (I) time from diagnosis to death before HSCT, (II) time from diagnosis to HSCT and (III) time from HSCT to death after HSCT. Most studied clinical variables have an immutable nature (e.g. the patients gender); in contrast, neutropenia and IA occur during follow-up and their effects may change over time. Therefore, these variables were used as time-dependent variables in the Cox model. As it is expected that the risk changes over time, a time-dependent model needs to be constructed to reliably account for IA and neutropenia as mortality indicators. For this purpose, time after the diagnosis of IA was divided into four time periods corresponding to the first, second and third month after diagnosis and the time period beyond (more than 90 days after). STATA (StataCorp. 2012. Statistical Software, Release 12.0) was used to perform all analyses. To construct the graphs, results from the analyses were imported in GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

Figure 1. Phases of treatment of haematological malignancy corresponding to analytical models estimating the probability of death after diagnosis of invasive aspergillosis



Legend: Arrows represent possible pathways during treatment for haematological malignancy. Numbered pathways correspond to the relevant models in table 2 and figure 3.

RESULTS

Study population

The cohort of patients receiving remission-induction therapy with the goal of attaining HSCT for AML or high-risk MDS consisted of 173 patients (table 1). Of these patients, 94 were

Table 1 Baseline characteristics of all included patients

Variable	Values (n, %)*
Number of patients	173
Number of treatment episodes	190
Number of cases of IA	55 (32)
Possible (% of total IA)	20 (36)
Probable (% of total IA)	33 (60)
Proven (% of total IA	2 (4)
Localisation of IA	
Only pulmonary	51 (93)
Only sinusoidal	2 (4)
Disseminated	2 (4)
Age (years), median (IQR)	54 (48-63)
Age >60	70 (40)
Sex (male)	94 (49)
Disease characteristics	
Secondary AML	12 (7)
Relapsed AML	27 (16)
Poor or Very poor prognosis[#]	92 (53)
>1 Remission-Induction-course	41 (24)
Total previous lung disease^	28 (16)
Previous pneumonia	17 (10)
COPD	8 (5)
Asthma	3 (2)
Pulmonary embolism	2 (1)
Lung cancer or metastases	1 (1)
Diabetes	14 (8)
Medication use	
G-CSF	36 (20)
Corticosteroids	69 (40)
Duration of neutropenia[$] (days), median (IQR)	54 (30-69)

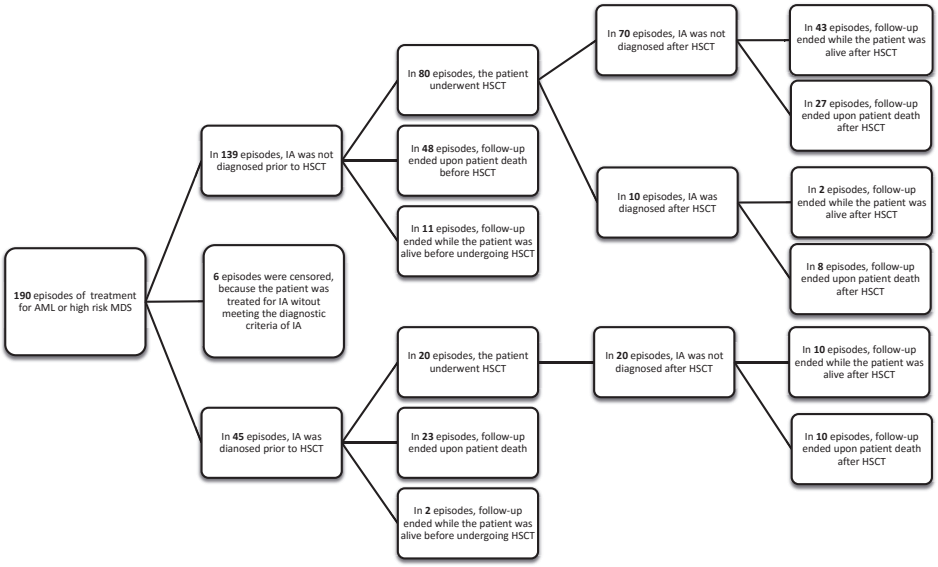
Legend: IA denotes invasive aspergillosis; 95%CI: 95% confidence interval; IQR: interquartile range; COPD: chronic obstructive pulmonary disease; G-CSF: granulocyte-colony stimulating factor; AML: acute myeloid leukaemia. *: Unless indicated otherwise. #: Risk classification according to national HOVON-102 protocol, ^: Lung disease defined as any lung disease stated in the patient's medical history (one patient could fall in more than one category of lung disease) $: Neutropenia defined as a blood granulocyte count <500/µL.

male and the median age was 54 years. In the pre-transplant period, patients that received mould-active antifungal agents for another reason than treatment of at least possible IA were censored at the moment of start of therapy (n=6). Reasons for the use of fungicidal drugs for other purposes than treatment of IA were the following: chemoprophylaxis for IA (n=1), aborted empirical treatment (n=4) and invasive fungal infection other than IA (n=1). IA was diagnosed in 55 patients and 112 patients (contributing 129 episodes) never experienced IA during follow up. IA occurred in 45 patients before transplantation, and in 10 patients after transplantation. None of the patients developed IA more often than once during the study period. The incidence of IA did not increase over the years during the study period (Chi^2: p= 0.095). In 12 (22%) cases either sputum or BAL samples yielded a positive culture. Fungal cultures displayed an usual distribution of *Aspergillus* spp.: eleven *A. fumigatus* and one *A. flavus* were identified. One cultured *A. fumigatus* was found to be resistant for itraconazole, posaconazole and voriconazole. As some patients developed a relapse of the haematological malignancy within the period of study, all 173 patients contributed a total of 190 episodes of treatment to the study.

Survival data

HSCT was performed after 100 (54%) of 184 episodes of remission-induction chemotherapy. In a total of 71 episodes (39%) the patient died prior to HSCT. For the remaining 13 episodes (7%), patients were not transplanted and still alive at the end of the period of study (figure 2).

Figure 2. Study cohort



Legend: AML denotes acute myeloid leukaemia, MDS myelodysplastic syndrome, IA invasive aspergillosis, HSCT haematopoietic stem cell transplantation.

Table 2 shows the increase in hazard after the diagnosis of IA, after adjusting for use of granulocyte-colony stimulating factor (GCSF), age, sex, WHO haematological risk category, number of chemotherapy courses needed to accomplish complete remission, previous lung disease, secondary or relapsed haematological disease and the presence of neutropenia. The different models correspond to the pathways visualised in figure 1: Model 1 describes the hazard of death before HSCT, model 2 the hazard of reaching the point of HSCT, model 3 the hazard of death after HSCT. The relative effects of individual factors remained constant in the different post-IA time periods and different treatment phases.

Table 2 Adjusted hazard ratios for reaching the endpoint per analysis and for probability of death pre- and post haematopoietic stem cell transplantation after diagnosis of invasive aspergillosis and different co-variates

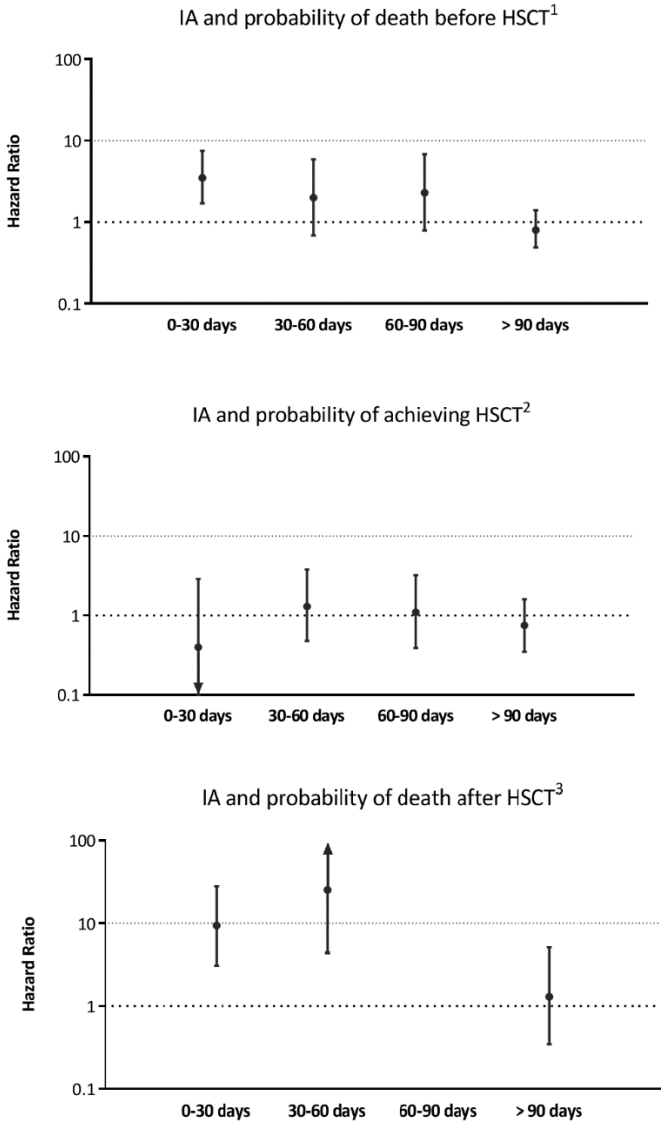
Variable	**Adjusted HR on death before HSCT (95%CI)**	**Adjusted HR on attaining HSCT (95%CI)**	**Adjusted HR on death after HSCT (95%CI)**
	(model 1)	**(model 2)**	**(model 3)**
Number of days after IA			
0 - 30 days	**3.5 (1.7 - 7.5)**	0.40 (0.05 - 2.9)	**9.4 (3.1 - 28.3)**
30 - 60 days	2.0 (0.69 - 5.9)	1.3 (0.48 - 3.8)	**25.5(4.4 - 146)**
60 - 90 days	2.3 (0.79 - 6.8)	1.1 (0.39 - 3.2)	NA*
> 90 days	0.80 (0.49 - 1.4)	0.75 (0.35 - 1.6)	1.3 (0.35 - 5.1)
Neutropenia[$] (per day)	1.1 (0.60 - 2.0)	0.67 (0.36 - 1.2)	12.3 (0.97 - 155)
Gender (male)	1.3 (0.92 - 1.9)	1.3 (0.86 - 2.0)	1.4 (0.79 - 2.5)
Age (per year)	**1.02 (1.00-1.03)**	**0.98 (0.97-0.99)**	1.00 (0.97-1.02)
Previous lung disease^	1.4 (0.87 - 2.3)	0.68 (0.37 - 1.3)	1.9 (0.82 - 4.5)
Use of G-CSF	0.78 (0.48-1.3)	**2.6 (1.6 - 4.1)**	0.66 (0.33-1.3)
Secondary AML	1.6 (0.81 - 3.1)	0.68 (0.27 - 1.7)	1.9 (0.66 - 5.4)
Relapsed AML	**3.0 (1.9 - 4.9)**	**0.49 (0.23 - 0.99)**	2.4 (0.93 - 6.0)
Haematological risk classification (per more unfavourable category)	**1.4 (1.1 - 1.9)**	1.0 (0.77 - 1.4)	1.1 (0.69 - 1.7)
Number of courses before complete remission (per course)	**1.6 (1.1 - 2.3)**	**0.56 (0.35 - 0.89)**	1.3 (0.66 - 2.8)

Legend: IA denotes invasive aspergillosis; HSCT: haematopoietic stem cell transplantation; HR: hazard ratio; 95%CI: 95 confidence interval; G-CSF: granulocyte-colony stimulating factor; AML: acute myeloid leukaemia. *: No events in this group ^: Lung disease defined as any lung disease stated in the patient's medical history, e.g. COPD or previous pneumonia; [#]: Risk classification according to national HOVON-102 protocol; [$]: Neutropenia defined as a blood granulocyte count <500 neutrophilic granulocytes/μL.

The HRs for survival with 95% confidence intervals for different time periods after the diagnosis of IA as presented in table 2 are shown graphically (figure 3) per different treatment phase. Shortly after diagnosis of IA the hazard of mortality both before and after HSCT is increased. This effect decreased over time and after 1 month in the pre-transplant phase, or after 2 months in the post-transplant phase, a significant effect is no longer observed. The

probability to receive stem cell transplantation was not significantly altered by the occurrence of IA as shown in the second model (figure 3).

Figure 3. Adjusted hazard ratios per analysis and relevant endpoints pre- and post haematopoietic stem cell transplantation; after diagnosis of invasive aspergillosis



Legend: AML denotes acute myeloid leukaemia, MDS myelodysplastic syndrome, IA invasive aspergillosis, HSCT haematopoietic stem cell transplantation.

DISCUSSION

In this study, we applied a novel approach to study the association of IA and mortality over time. This association is complicated by several confounding factors and is not only challenging to appraise from an analytical perspective, but is also difficult to interpret for the clinician at the bedside of the individual patient. Mortality caused by IA is typically due to haemorrhage of lung lesions, insufficient oxygenation or dissemination to other vital organs such as the brain (15, 16). In clinical practice however, only a relatively small number of patients with IA dies from an obvious IA-related cause. This proportion further decreases over time from the day of diagnosis of IA (3, 17, 18). Different studies that assessed IA mortality in the past concluded that the directly attributable mortality is relatively low when compared to all-cause mortality following infection (2, 19-22). Factors associated with the development of IA are also predictors for an unfavourable outcome, even so in the absence of IA (7, 9, 23, 24). In some cases, the development of IA can be a phenomenon accompanying a deteriorating physical condition due to other fatal complications (e.g. pseudomonas sepsis) or be part of a polymicrobial infection, as has been reported in different cohort studies (25, 26). These previous findings, combined with the high baseline mortality of haematological patients, could easily give rise to overestimation of the IA-attributable mortality. As a result, the appraisal of the benefit of prophylactic or therapeutic antifungal strategies is complicated.

We found that the association between IA and mortality remains strong even after accounting for risk factors attributable to the unfavourable underlying disease. Both before and after HSCT, the mortality hazard is substantially increased shortly after the diagnosis of IA. However, within a relatively short period of time the effect of IA on the risk of death decreased. The mortality rates may then be directed by other host factors. A decrease of transplantation probability was observed within the first month after diagnosis of IA, but the effect was not statistically significant. This suggests that occurrence of IA might deter the clinician to proceed with a planned HSCT in the initial phase. Delay of HSCT for any reason lowers the probability of complete remission of haematological malignancy and is therefore undesirable (27, 28).

The results stress the importance of effective management in the initial phase (i.e. the first month) after diagnosis. In light of increasing resistance rates of *Aspergillus* spp. worldwide (29), determination of azole susceptibility should be pursued quickly to ensure the use of adequate therapy as soon as possible. This especially holds true in areas with high resistance rates. The applicability of the results is restricted by the selection of a population that consists of patients that have a high risk of IA. Other populations at risk for IA, such as solid-organ transplant recipients, develop IA in the background of a very different risk profile and are not comparable to the population described in this study. The study is somewhat limited by the s of the cohort, but despite the retrospective acquisition of the data, all episodes were well documented in our hospital system.

According to the methods described, we have included patients with possible IA because the results would best reflect a "real life clinical setting". Although the a priori chance of the presence of IA is theoretically smaller in the group of possible IA, the clinical consequences do not largely differ in terms of treatment and follow-up measures. As Herbrecht et al explain in their study implementing the updated 2008 EORTC definitions for IA (30), the diagnosis of possible IA in the clinical setting very likely corresponds to presence of disease, albeit usually in an earlier stadium with a more favourable prognosis. The inclusion of possible IA in this study contributes to the overall applicability of the results to the clinical situation.

A major strength of this study is the availability of day-by-day data over the entire course of the haematological disease and its integration in a time-dependent model. The presence of factors strongly associated with both mortality and the occurrence of IA demands a detailed parsing of all possible confounding factors for an accurate measurement of effect. Secondly, the study has a large number of participants without primary prophylaxis in the pre-transplantation period; this provides a mortality risk that does not rely on break-through infection or prophylaxis-intolerance. Future research would benefit from a protocolised method to establish the presence of co-infection and the cause of death. This would allow more precise calculation of attributable mortality rates of IA. Though difficult to achieve, high autopsy rates providing a more verifiable way to establish the cause of death, which would also be a valuable addition to future studies.

In conclusion, a detailed survival model of patients with AML or high risk MDS was established separately for all different treatment phases, focussing on the impact of IA on mortality in this population. The results show that the effect of IA on mortality is initially substantial. However, while an increased risk of death was significantly present in the first and second month after diagnosis of IA, no unfavourable association with mortality was observed in the later course of treatment. Thus, IA should not be considered as an independent determinant in long-term survival of patients treated for AML or high-risk MDS.

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Contributions: RP and MB initiated the study and were involved in the design. RP and PB contributed to the data collection. SC contributed to the study design and statistical analysis. All authors were actively involved in manuscript preparation and have read and approved the final manuscript.

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Chapter 5

Managing invasive aspergillosis in haematological patients in the era of resistance PCR and increasing triazole resistance: a modelling study of different strategies

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ABSTRACT

Objectives: Triazole resistance in *Aspergillus* spp. is emerging and complicates prophylaxis and treatment of invasive aspergillosis (IA) worldwide. New polymerase chain reaction (PCR) tests on bronchoalveolar lavage (BAL) fluid allow for detection of triazole-resistance on a genetic level, which opened up new possibilities for targeted therapy. In the absence of clinical trials, a modelling study delivers estimates of the added value of resistance detection with PCR and which empiric therapy would be optimal when local resistance rates are known.

Design: We performed a decision-analytic modelling study based on epidemiological data of IA, extended with estimated dynamics of resistance rates and treatment effectiveness. We compared six clinical strategies that differ in use of PCR diagnostics (used versus not used) and in empiric therapeutic choice in case of unknown triazole-susceptibility: voriconazole, liposomal amphotericin B (LAmB) or both. Outcome measures were proportion of correct treatment, survival and serious adverse events.

Results: Implementing *Aspergillus* PCR tests was projected to result in residual treatment-susceptibility mismatches of <5% for a triazole resistance rate up to 20% (using voriconazole). Empiric LAmB outperformed voriconazole at resistance rates higher than 5-20%, depending on PCR use and estimated survival benefits of voriconazole over LAmB. Combination therapy of voriconazole and LAmB performed best at all resistance rates but the advantage over the other strategies should be weighed against the expected increased number of drug related serious adverse events. The advantage of combination therapy over LAmB monotherapy became smaller at higher triazole-resistance rates.

Conclusions: Introduction of current *Aspergillus* PCR tests on BAL-fluid is an effective way to increase the proportion of patients that receive adequate targeted therapy for IA. The results indicate that close monitoring of background resistance rates and of adverse drug events are important to attain the potential benefits of LAmB. The choice of strategy ultimately depends on the probability of triazole-resistance, the availability of PCR and individual patient characteristics.

INTRODUCTION

Invasive aspergillosis (IA) is an opportunistic fungal infection with rising incidence among various patient populations. Patients treated for haematological malignancy with intensive chemotherapy or haematopoietic stem cell transplantation (HSCT) are the population with the highest risk of developing IA and often receive antifungal chemoprophylaxis throughout treatment. Despite the use of chemoprophylaxis, incidence rates in this population remain substantial and IA continues to cause significant morbidity and mortality (1). Developments in applicability of PCR diagnostics as well as the increasing incidence of antifungal resistance worldwide urgently calls for optimization of the strategies for managing IA (2, 3).

Aspergillus triazole resistance rates in Northwestern Europe are reported to be amongst the highest in the world, varying between 8-15% and showing an increasing trend over time. Multiple reports of worldwide emerging triazole-resistance confirm that the problem is expanding on a global scale. This is presumably due to the high mobility of *Aspergillus* spores and increased awareness (3, 4).

When inadequately treated with triazoles, the mortality of patients infected with triazole resistant *Aspergillus spp*. is reported to be high as 88% (5, 6). Hence, triazole resistance will increasingly complicate the efficacy of chemoprophylaxis and therapeutic management of IA and is associated with a higher mortality.

Due to the limited sensitivity of culture with subsequent susceptibility testing, triazole-susceptibility is often unknown, which creates a clinical dilemma. Evidence of superior efficacy of triazoles versus amphotericin B has been demonstrated in the trial by Herbrecht et al. in 2002 (7). Since then, no head-to-head comparisons between voriconazole versus any formulation of amphotericin B have been investigated under randomized conditions. Thus, voriconazole has remained the primary treatment choice in international guidelines (8). However, the risk of treating disease caused by triazole-resistant *Aspergillus* with a triazole could offset the potential survival benefit in the overall population. The importance of initiating the correct treatment as soon as possible is supported by survival data that show that mortality is highest within the first phase of treatment (9-11).

In recent years, polymerase chain reaction (PCR) on bronchoalveolar lavage (BAL)-fluid opened up new possibilities in the diagnosis of IA. In addition to providing a higher sensitivity and specificity in BAL-based diagnostics, this technique is now able to detect triazole resistance on a genetic level by analysis of CYP51-gene mutations. Thereby, phenotypical susceptibility testing on a positive culture is no longer the only way to demonstrate the presence of antifungal resistance (12). Effectively implementing this new strategy facilitates the use of rapid targeted therapy. However, setting up a randomized diagnostic trial using PCR-based diagnostics in a setting of triazole resistance would need a high number of participants and many years to complete.

Hence, our first aim is to combine available data of previous study outcomes and current test characteristics in a simulation model to assess the potential impact of PCR diagnostics and the selective use of voriconazole and liposomal amphotericin B (LAmB) on mortality. We explore three different strategies that reflect the current clinical landscape. The second aim of this study is to explore which information would be most useful to collect to reduce the uncertainties regarding the survival benefit of voriconazole versus LAmB under different resistance rates in a comprehensive model.

DESIGN

Population

The modelling study focused on a population comprised of patients undergoing treatment for a haematological malignancy. The main assumptions were that a clinical suspicion of IA caused by *Aspergillus* fumigatus was present, and a BAL was performed in an attempt to establish the diagnosis. Polyene resistance was presumed to be absent. The population consisted of 1000 patients, a number that a large multicentre study might reach within several years. PCR results were supposed to be available within 48 hours, thus preventing a relevant delay in susceptibility testing.

Strategies

All patients in this population were subjected to six different strategies of diagnosis and treatment. In all six strategies (table 1), patients with proven susceptible IA were treated with voriconazole monotherapy and patients with proven resistance were treated with LAmB monotherapy. The strategies differ in empiric therapy used in case of unknown azole-susceptibility (strategy 1 uses voriconazole (VOR), strategy 2 uses LAmB, and strategy 3 uses a combination of both (COMB)), as well as the use of diagnostic PCR (strategies 1A, 2A, 3A use diagnostics without PRC, whereas strategies 1B, 2B, 3B use PCR for resistance detection).

Table 1: Overview of the diagnostics and treatment used in six different strategies for managing invasive aspergillosis.

Strategy	PCR for resistance detection	demonstrated azole-resistance	demonstrated azole-sensitivity	Unknown Azole-Sensitivity
1A	No	LAmB	Voriconazole	Voriconazole
1B	Yes	LAmB	Voriconazole	Voriconazole
2A	No	LAmB	Voriconazole	LAmB
2B	Yes	LAmB	Voriconazole	LAmB
3A	No	LAmB	Voriconazole	Combination therapy voriconazole + LAmB
3B	Yes	LAmB	Voriconazole	Combination therapy voriconazole + LAmB

Legend: PCR denotes polymerase chain reaction, LAmB liposomal amphotericin B.

Outcome measures

The relevant outcomes were: the proportion of patients with triazole resistant IA that received the correct treatment (i.e. LAmB), and conversely, the percentage with treatment mismatch, as well as the survival and the occurrence of serious adverse events. Given the rarity of LAmB resistance in *Aspergillus* fumigatus, therapy mismatch was defined in this study only as voriconazole in case of triazole resistance. LAmB was considered correct treatment regardless of azole susceptibility. Possible survival disadvantage of LAmB compared to voriconazole in case of azole susceptibility was addressed in the model.

Decision tree

A decision tree that reflects the diagnostic pathway for the six strategies has been constructed (figure 1). The path each simulated patient takes was determined by probabilities for each step in the pathway. If the galactomannan test is negative, a positive result on the *Aspergillus* PCR is highly improbable, and these exceptions were not included in the model (12-14). The outcome of culture is displayed before the outcome of the PCR, although chronologically, the reverse would be true. The possible benefit of earlier diagnosis by PCR was not taken into account. However, the flowchart order of culture and PCR has no effect on the model outcomes. The displayed order demonstrates the added value for PCR in culture negative patients most clearly.

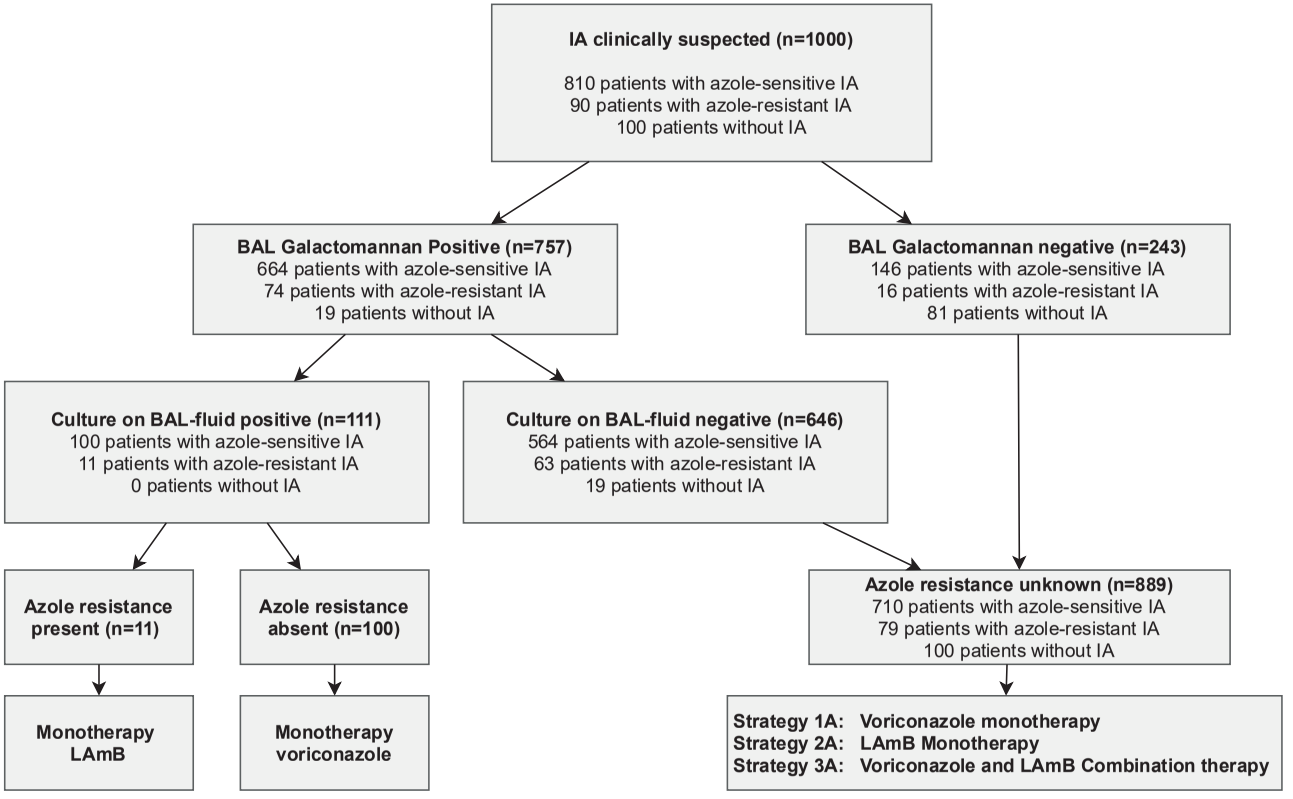
Literature review

To obtain realistic characteristics of the performance of diagnostic tests and the outcome of disease, a literature review was conducted. The values of probabilities for different steps in the diagnostic pathway were extracted from published meta-analyses, systematic reviews or randomized controlled trials. When the values of these probabilities could not be determined precisely from the literature, a sensitivity analysis for this value was used to explore the impact of this uncertainty on the outcome of the simulation model. The sensitivity and specificity values as well as the accuracy of resistance detection was extracted from two recent studies that evaluated PCR techniques in at least 100 clinical cases. Sensitivity of PCR varies widely depending on the DNA isolation and amplification methods and therefore only commercial real-time assays directing CYP51 mutations were included. Notable studies with smaller numbers of included patients show similar values (14, 15).

Parameter values and sensitivity analysis

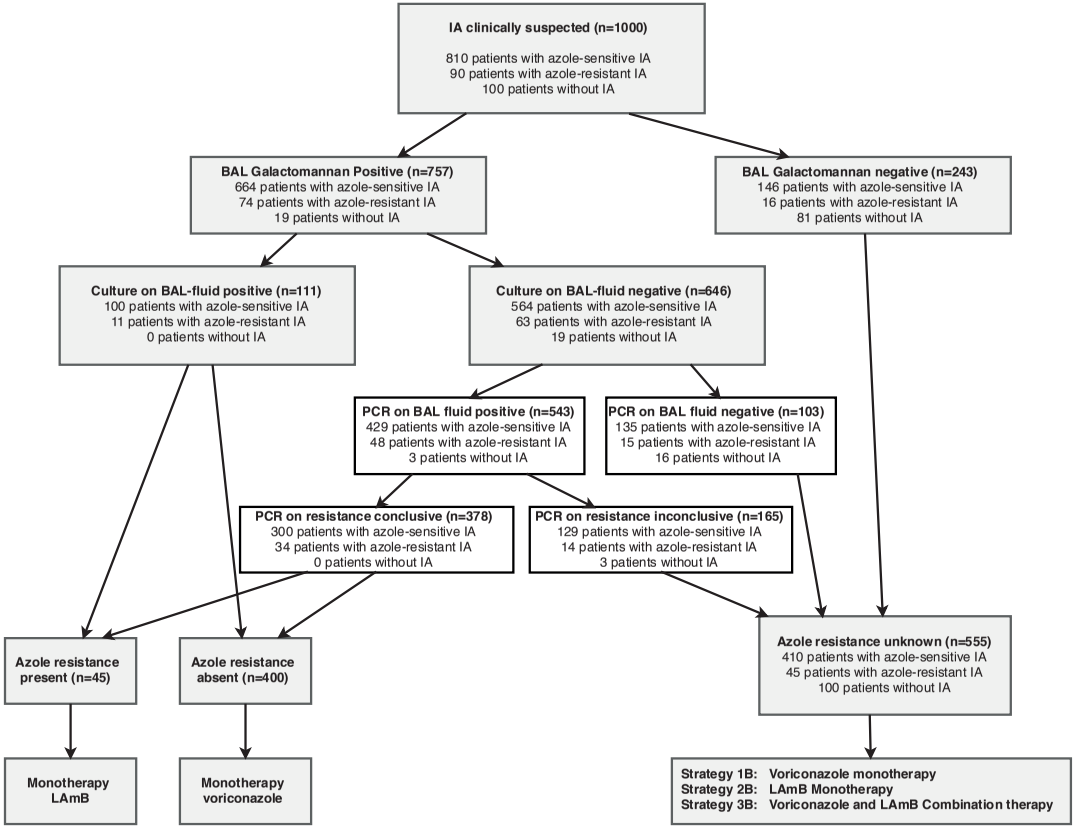
Based on the literature review, the probabilities were set to values as indicated in Table 2. To reflect the uncertainty in the survival between treatment with voriconazole and LAmB, three different scenarios were explored: (1) the mortality of patients treated with LAmB is consistent with the rates of conventional amphotericin-b deoxycholate as extracted from Herbrecht et al. (10) (0.371); (2) the mortality of patients treated with LAmB is consistent with the rates from the AmBiload study (0.280) (11, 16); (3) the mortality of patients treated with LAmB

Figure 1. Treatment flowcharts for the six different treatment strategies
A. Treatment flowchart for all strategies for managing invasive aspergillosis without using a PCR, representing strategy 1A, 2A and 3A



Legend: IA denotes invasive aspergillosis, BAL bronchoalveolar lavage, LAmB liposomal amphotericin B. Patients follow the steps in the flowchart according to the characteristics presented in table 2.

B. Treatment flowchart for all strategies for managing invasive aspergillosis using a PCR on BAL-fluid, representing strategy 1B, 2B and 3B



Legend: IA denotes invasive aspergillosis, BAL bronchoalveolar lavage LAmB liposomal amphotericin B, PCR polymerase chain reaction. Patients follow the steps in the flowchart according to the characteristics presented in table 2.

is estimated to be an aggregate of scenario (1) and (2), set at 0.325. To explore the impact of strategies over a realistic range of triazole resistance rates (2, 3, 6, 17, 18), we varied resistance rates from 5%, increasing in steps of 5% up to a triazole resistance rate of 30%.

Table 2: Overview of literature used to specify different patient, test and treatment characteristics.

Parameter	Literature used	Value
Sensitivity of clinical suspicion	*NA*	NA
Specificity of clinical suspicion	*NA (model assumption)*	0.90
Sensitivity of BAL Gm-assay	*Leeflang[27] 2015*	0.82*
Specificity of BAL Gm-assay	*Leeflang[27] 2015*	0.81*
Sensitivity of culture	*Barton[28] 2013*	0.15 (0.10 -0.58)
Specificity of culture	*Barton[28] 2013*	NA
Sensitivity of PCR	*Chong[12] 2016, Montesinos[13] 2017*	0.76 (0.66-0.86)
Specificity of PCR	*Chong[12] 2016, Montesinos[13] 2017*	0.83 (0.80-0.86)
Probability of successful susceptibility determination by PCR	*Chong[12] 2016*	0.70
VOR 12 week CFR (triazole-sensitive)	*Herbrecht [10] 2002 (updated[10] 2015)*	0.245
VOR 12 week CFR (triazole-resistant)	*Van der Linden 2011, Steinmann [6] 2015*	0.88*
AmB-d 12 week CFR	*Herbrecht [10] 2002 (updated[10] 2015)*	0.371
LAmB 12 week CFR	*Cornely[11] 2007*	0.280
VOR risk of serious AE	*Herbrecht [10] 2002 (updated 2015)*	0.05
LAmB risk of serious AE	*Botero Aguirre[29] 2015*	0.128*

Legend: NA denotes not available; Gm galactomannan; BAL bronchoalveolar lavage; VOR voriconazole; CFR case fatality rate; LAmB liposomal amphotericin B; PCR polymerase chain reaction. Amb-d amphotericin b deoxycholate; AE adverse event. *Study population not limited to haemato-oncological patients but consisting of different immunocompromised patients.

Statistical analysis

STATA (StataCorp. 2012. Statistical Software, Release 12.0) was used to perform all analyses and to construct the graphs. The syntax that was used to build the database and to perform the analyses can be found in the supplemental data (supplement 1, published online).

RESULTS

Literature review and model parameters

The results of the literature review are summarized in table 2. All studies only included patients that were being treated for a haematological malignancy unless stated otherwise. In case of different value parameters extracted from multiple relevant studies, an aggregate mean value has been used. Herbrecht et al. (7, 10) performed the only randomised trial that has investigated

a head-to-head comparison between voriconazole versus a formulation of amphotericin B. However, there is ongoing debate about the applicability of the results in the current clinical landscape (7, 11, 16). Because the study by Herbrecht et al. compared voriconazole with amphotericin B deoxycholate instead of the currently used liposomal formulation, it has been argued that the survival benefit of voriconazole is in fact smaller. The AmbiLOAD trial (11) has provided a randomized study population that has been treated with LAmB. As argued by Denning et al. (16), one could compare the results from both studies and conclude that there is no difference in survival between voriconazole and LAmB.

There was no consistent data that allowed for the estimation of survival of patients with IA primarily treated with both voriconazole and liposomal amphotericin B, survival in strategy 3A and 3B was therefore presumed to be equal to that of voriconazole for a triazole-sensitive IA and to that of LAmB for a triazole-resistant IA. Clinical evidence for an antagonistic or synergistic effect of the combination of an polyene and a triazole is absent (19, 20).

Model outcomes

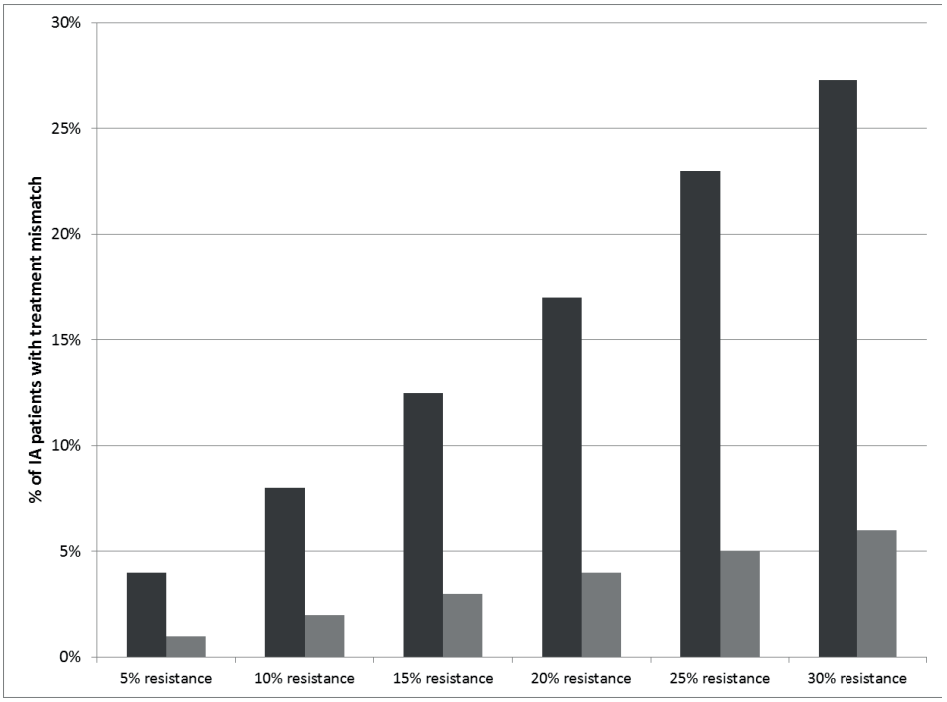
Each of the 1000 patients in the population followed the decision tree for each scenario. Numbers of patients in each step are shown in figure 1. The numbers of patients in each step are based on the parameters presented in table 2. For example, the number of patients with IA that get a positive BAL-galactomannan (664 triazole-sensitive plus 74 triazole-resistant IA patients) is computed as the sensitivity of the BAL-galactomannan test (0.82) multiplied by the total number of patients with IA (810 triazole-sensitive plus 90 triazole-resistant IA patients). The same goes for the patients without IA, using 1 minus the specificity (1 minus 0.81 = 0.19), resulting in 19 patients without IA and a false positive test.

Based on the parameters aggregated in table 2, we have simulated the effects on our primary outcomes: the proportion of patients with triazole resistant IA that received the correct treatment (i.e. LAmB), case fatality rate and the occurrence of serious adverse events.

Correct treatment

Using the targeted strategies in which PCR diagnostics were implemented, more patients received LAmB for a triazole-resistant IA and voriconazole for triazole-sensitive IA. The higher the rate of triazole-resistance, the larger the benefit of the targeted strategy 1B on the decrease of treatment-mismatch (figure 2). If PCR is not used, a linear increase in the number of patients incorrectly treated with voriconazole is expected when resistance rates are rising. Up to a triazole-resistance percentage of 20% of all IA occurrences, this number can be reduced below 5% by implementation of PCR-based triazole-susceptibility testing. Not displayed in this graph are strategy 2A, 2B, 3A and 3B, as these strategies include the use of LAmB in case of unknown triazole sensitivity and will thereby always guarantee adequate treatment of triazole resistant IA.

Figure 2. Triazole-resistant invasive aspergillosis treated with voriconazole in strategy 1A (no PCR) vs 1B (PCR) as a percentage of all patients with invasive aspergillosis.



Legend: VOR denotes voriconazole; PCR polymerase chain reaction; IA invasive aspergillosis. Treatment mismatch is defines as an azole resistant invasive aspergillosis treated with voriconazole. Details of different strategies can be found in table 1.

Survival

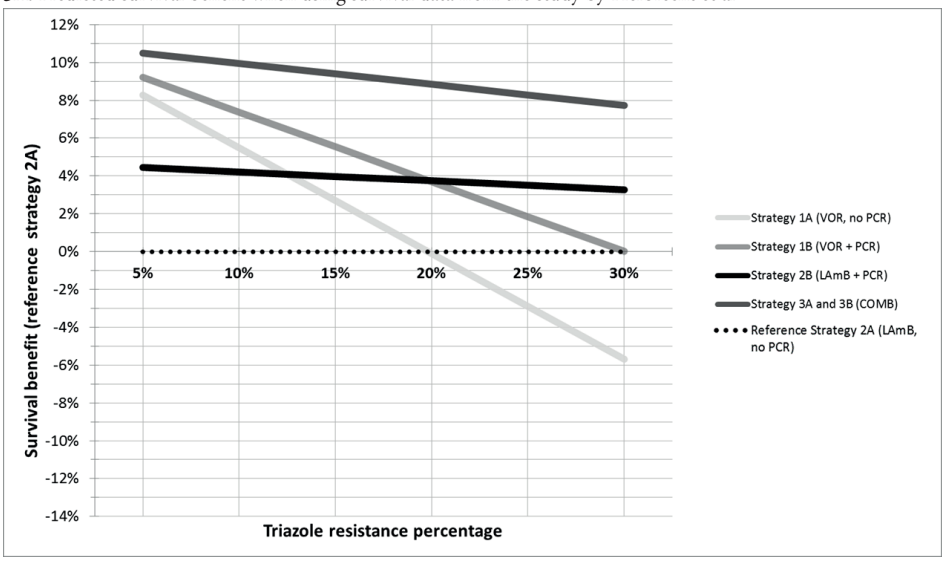
As survival in strategy 2A is almost constant among the different imputed resistance rates (varying less than 0.5% between the outer values), this strategy was most suitable as reference category. The absolute survival benefit of the other strategies when compared to strategy 2A (LAmB in case of unknown triazole-susceptibility, no use of PCR) is displayed in figure 3A-C.

Survival improves in strategy 1B (VOR + PCR) compared to 1A (VOR, no PCR) due to the decreased proportion of patients with triazole-resistant IA who are treated with voriconazole (see also figure 2). The higher the rate of triazole-resistance, the larger the benefit of the PCR diagnostics was in the simulated population.

Strategy 2A and 2B (LAmB) are inferior to strategy 1A and 1B (VOR) at low resistance rates, and only provide better survival if the resistance rates are high enough. Depending on the assumed superiority of VOR over LAmB for azole-susceptible IA, the tipping point of superiority is around 20% (figure 3A, Herbrecht data) to only 5% (figure 3C, AmbiLoad data).

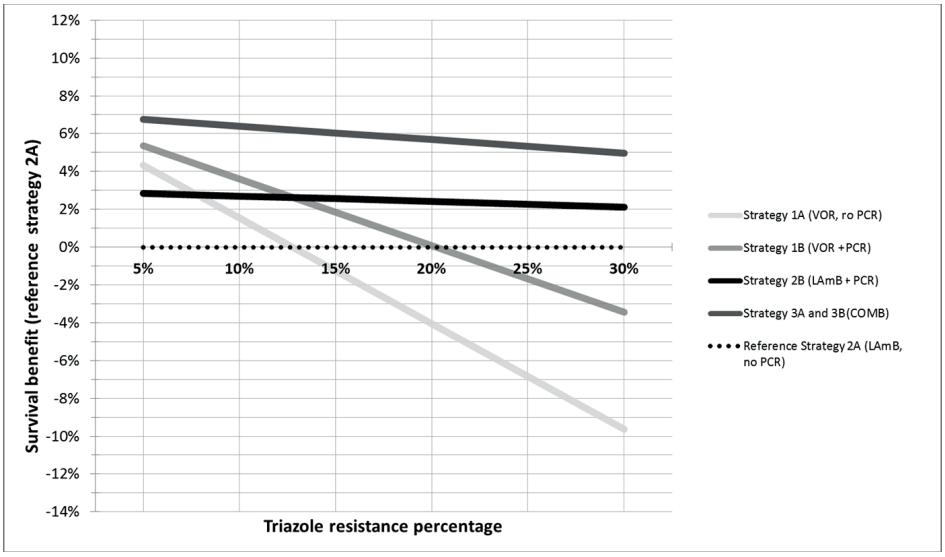
Figure 3. Predicted absolute survival benefit of different clinical strategies compared to strategy 2A (liposomal amphotericin B in case of unknown triazole-susceptibility and no use of PCR resistance detection) in patients with invasive aspergillosis.

3A: Predicted survival benefit when using survival data from the study by Herbrecht et al



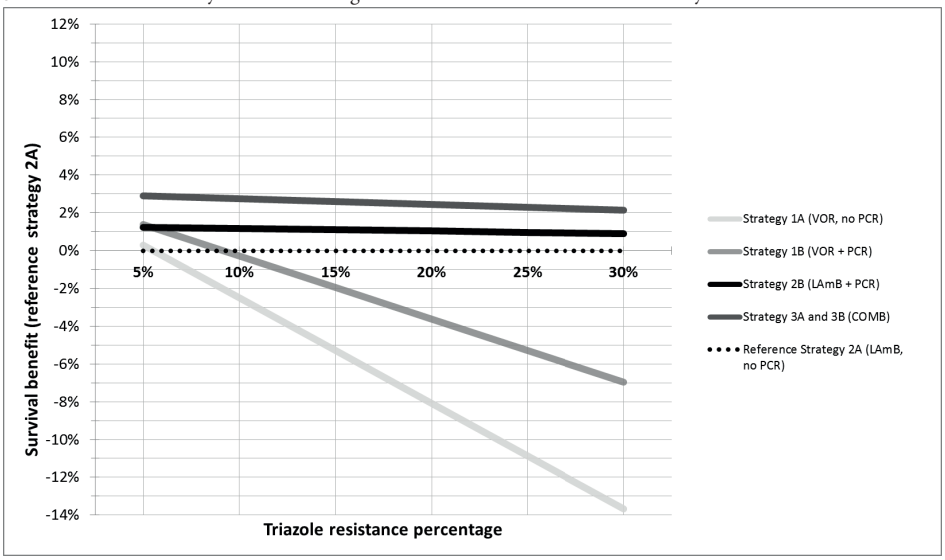
Legend: VOR denotes voriconazole; LAmB liposomal amphotericin B; PCR polymerase chain reaction. Details of different strategies can be found in table 1.

3B: Predicted survival rates when combining survival data from the AmbiLOAD study and the study by Herbrecht et al.



Legend: VOR denotes voriconazole; LAmB liposomal amphotericin B; PCR polymerase chain reaction. Details of different strategies can be found in table 1.

3C: Predicted case fatality rates when using survival data from the AmbiLOAD study



Legend: VOR denotes voriconazole; LAmB liposomal amphotericin B; PCR polymerase chain reaction. Details of different strategies can be found in table 1.

Strategy 3A and 3B (COMB) yield the best survival for all resistance rates. The use of PCR in strategy 3B only benefits the rates of adverse events due to increased use of targeted monotherapy, so no difference between mortality was found between strategy 3A and 3B. Therefore, the results of these strategies are shown as a single line (figure 3A-C).

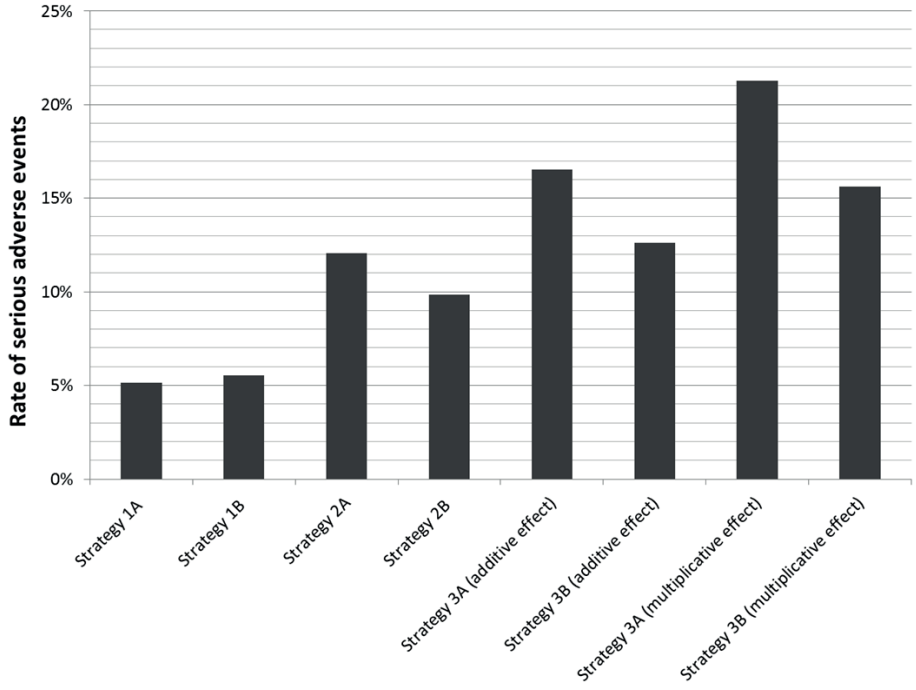
Above 20% resistance rates we found a clear inferiority of strategies that use voriconazole in case of unknown triazole-susceptibility (strategy 1A and 1B); however, the advantage of the other strategies must be weighed against the expected increased serious adverse event rates. Notably, the advantage of combination therapy versus LAmB monotherapy becomes increasingly smaller at higher triazole-resistance rates. At 15% percent resistance, the survival difference between these strategies is around 1.5% percent using only the data from the AmBiLoad trial to calculate survival rates (figure 3C). At lower resistance rates (less than 10%), strategy 1B remained within a range of 3% survival inferiority when compared to combination therapy, even when only the survival data from the AmBiLoad study were used (figure 3A).

Toxicity

Strategies 3A and 3B (COMB) had the highest rates of serious adverse events as they often combine both toxic forms of therapy (figure 4). When comparing strategy 1A and 1B (VOR), patients who were tested with a PCR suffered more nephrotoxicity as more patients are treated with LAmB whereas in strategy 2 and 3, PCR decreased toxicity by reducing unnecessary use of LAmB. We explored both an additive and multiplicative effect of therapy on serious adverse

events. This reveals that the rate of serious adverse events may be even higher if there is a multiplicative effect of therapy on toxicity. Resistance rate increase did not have an important effect on adverse event rates and are not shown in the graph. At most, a 1% difference in adverse event rate was found between the outer values of the imputed resistance rates. The weighing of resistance rates against survival rates are important as the survival benefit is smaller at low resistance rates but the occurrence of adverse events remains relatively stable.

Figure 4: Predicted rates of serious adverse events in six different clinical strategies using both an additive and a multiplicative model to predict outcomes of combination therapy



Legend: Details of different strategies can be found in table 1**.**

DISCUSSION

Summary

Our study provides a comprehensive insight in the strengths and weaknesses of different strategies of antifungal chemotherapy for IA. Introduction of species- and CYP51-gene PCR of BAL-fluid seems to provide an effective way to increase the number of patients that receive targeted therapy for IA. The current limitations in sensitivity and specificity leave around half of all patients in which antifungal sensitivity remains unknown, thus necessitating a well informed choice for this large group of patients. Strategies that incorporate the use of LAmB

in case of unknown triazole-susceptibility are more effective when the background resistance rates are higher and when the true difference of treatment effectiveness between voriconazole and LAmB is smaller. The occurrence of antifungal related serious adverse events is higher in a strategy in which more patients receive LAmB. This holds particularly true for a strategy that combines LAmB and voriconazole, although the exact number of adverse events is hard to quantify due to insufficient data.

Validity of the model assumptions

The performance of PCR in the diagnosis of IA is only recently explored and the experience with the diagnostic value in clinical practice is limited (12). The difference between the A and B variants of the strategies (with or without PCR) is largely dependent on the data from a few studies published after the introduction of this diagnostic method (12-14). More recent findings suggest the initial findings may be too optimistic (21). On the other hand, research devoted to the combination of PCR with other diagnostic assays also show promising results (22-24). Of note, the techniques that were included in the literature review only cover a single resistance locus; changes in epidemiology of the resistance mechanisms could potentially decrease the benefit of PCR for susceptibility testing. Moreover, these studies were not powered to provide an estimate for the sensitivity of the PCR for the detection of resistance.

Another important factor in the model is the a priori chance of the presence of a clinical significant fungal infection in a patient with a positive HR-CT scan. Our results are in particular dependent on this number; if this chance is lower, a lot of patients would unnecessarily be exposed to the toxic effects of LAmB or combination therapy and the survival differences would be smaller. It is difficult to provide a reliable estimate of this chance, as the positive HR-CT itself justifies the diagnosis of a possible IA in an appropriate host. Our only source could be the results from autopsy studies (25, 26). The absence of IA at an autopsy does not rule out the absence of IA at the moment of the initiation of treatment however. Hence, using data from autopsy studies would underestimate this probability. In clinical practice, it is assumed that a positive HR-CT scan in absence of more plausible differential diagnostic entities is a fairly certain marker of the presence of disease. Therefore, for the purpose of our study, a probability of 0.90 has been implemented in the model.

It should be noted that the numbers on which the estimates of resistance percentages are based on, are mostly derived from data of probable and proven IA, and could therefore be an overestimation of the overall resistance percentage. The difference in resistance rates between continents, regions and even individual hospitals are an important aspect in the interpretation of our study results for a policy in clinical practice. Additionally, polyene resistance is not taken into account. Hospitals that are experiencing a substantial burden of polyene resistant species should expect that the benefits of LAmB are lower than in the simulated population.

We have incorporated as many relevant factors as possible in the model in order to take into account all aspects of the treatment landscape in which the clinical problem takes place.

However, one important factor that is worth mentioning is the absence of a strategy that incorporates the use of echinocandins. Several studies are available on the incorporation of echinocandins in the treatment of IA. It is either used as standard or salvage therapy, as monotherapy or used in conjunction with a triazole or LAmB (27-29). Because these strategies are very diverse and are usually recommended as salvage therapy in international guidelines (8, 30), these strategies were not taken into account in our model.

Strengths and limitations

The strength of our study is the synthesis of evidence present in the current literature. Six different treatment strategies were compared at a range of resistance rates and alternative scenarios for therapy effectiveness. This allows researchers to select those study results relevant to the resistance rates in the population of interest, and this will provide a rationale for discussing an appropriate treatment strategy in their institution.

Our findings open perspectives for further research that will further support clinical decision making. First, it is possible to extend the scope by including relevant information on associated morbidity, quality of life and the costs of treatment and care. This would require reliable results on morbidity, quality of life and costs, and on the relation between IA, antifungal treatment and risk factors for invasive fungal disease. In absence of these reliable results, we have limited our study to treatment options. Second, it is possible to include alternative tests as they come available in the future to keep the results relevant in the ever-changing clinical landscape.

The main strength, as with all simulation studies, is the identification of those parameter values that are most valuable to get more accurate estimates of the impact of treatment. In our study, one of the most valuable parameters is the survival benefit of voriconazole as compared to LAmB. Only one large trial, conducted more than 15 years ago, has compared voriconazole directly to conventional amphotericin B. More recent research (11, 16) suggests that the difference on survival between the two therapies might not be as large as that observed in the study by Herbrecht et al. (10). To address this uncertainty, we have used three different scenarios of relative therapy effectiveness. This way, the validity of the model remains assured within each background assumption of this difference. Another parameter value that would be very informative is the rate of adverse events in combining voriconazole and LAmB use (strategies 3A and 3B). Experience with this strategy is very limited in clinical practice and it is not known if a synergistic or antagonistic antifungal effect exists when combining the two drugs (19, 20). Reversely, this also holds true for a potential interactive effect of the occurrence of serious adverse events (20, 31). The impact of the recent introduction of isavuconazole for the treatment of IA is not addressed by the model. As the first experience with this drug shows a potential effect in reduction of adverse events, implementing this could further increase the benefit of the triazole-class of antifungals over LAmB with regard to drug-related adverse events. Consequently, within the setting of combination therapy of LAmB and a triazole, isavuconazole could potentially remove some of the disadvantages of combining two antifungals

with regard to interactions and toxicity. Evidence suggests that hepatobiliary adverse events, as well as neurological, skin and eye disorders, are less common when using isavuconazole when compared to voriconazole. No effect in reducing mortality was found however. (32, 33)

Conclusions

The choice of the best strategy is largely dependent on the rate of triazole resistance. Among all modelled scenarios, strategies that combine voriconazole and LAmB yield superior survival. However, both lower resistance rates and lower difference in therapy effectiveness between the two classes of antifungals reduce the actual benefit of this strategy when compared to a strategy with monotherapy combined with PCR, while the high rate of expected adverse events remains constant. Implementation of resistance detection with PCR could reduce the adverse events rates if the patients switch to adequate monotherapy following conclusive results in susceptibility testing.

PCR may increase survival in settings where empiric voriconazole is used and may aid in reducing toxicity in settings with empiric LAmB. When estimating the survival benefit of voriconazole versus LAmB by combining the data from the AmbiLOAD (11) and the study by Herbrecht et al. (10), the percentage from which superiority of LAmB is achieved lies between 10% and 15%. However, therapy tailored toward the individual patient should always be pursued. For example, pre-existing nephropathy could discourage the clinician to treat with LAmB, or prolonged triazole exposure through prophylaxis could discourage treatment with voriconazole. Furthermore, clinical risk factors and co-morbidities could change the parameters on which our model is based, and subsequently the expected outcomes.

The model clearly shows that introduction of currently available commercial *Aspergillus* PCR tests on BAL-fluid is an effective way to increase the proportion of patients that receive targeted therapy for IA to obtain the optimal outcomes. Furthermore, it is apparent that close monitoring of background resistance rates and of adverse drug events are important to warrant that the expected benefits of LAmB at higher triazole resistance rates are actually realized.

Acknowlegement

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Competing Interests: None

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Chapter 6

Outpatient Parenteral Antifungal Therapy (OPAT) for invasive fungal infections with intermittent dosing of liposomal amphotericin B.

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ABSTRACT

Introduction: Triazole resistant *Aspergillus fumigatus* has been documented in many parts of the world. In the Netherlands, incidence is now above 10% and results in the need for long-term parenteral therapy with liposomal amphotericin B (LAmB). The long terminal half-life of LAmB suggests that intermittent dosing could be effective, making the application of outpatient antifungal therapy (OPAT) possible. Here, we report our experience with the use of OPAT for invasive fungal infections (IFI).

Methods: All adult patients treated with LAmB with a 2 or 3 times weekly administration via the outpatient departments in four academic tertiary care centres in the Netherlands and Belgium since January 2010 were included in our analysis. Patient characteristics were collected, as well as information about diagnostics, therapy dose and duration, toxicity, treatment history and outcome of the IFI.

Results: In total, 18 patients were included. The most frequently used regimen (67%) was 5mg/kg 3 times weekly. A partial response to the daily treatment prior to discharge was confirmed by CT-scan in 17 (94%) of patients. A favourable outcome was achieved in 13 (72%) patients. Decrease in renal function occurred in 10 (56%) cases but was reversible in all and was treatment limiting in 1 patient only. 100-day mortality and 1-year mortality after initiation of OPAT were 0% and 6%, respectively.

Conclusions: In a selected population, and after confirmation of initial response to treatment, our data support the use of OPAT with LAmB for treatment of IFI in an intermittent dosing regimen.

INTRODUCTION

Invasive fungal infections (IFI) are often life-threatening and occur predominantly in immunocompromised patients. After surviving the initial phase of infection, prolonged treatment with an antifungal agent is often necessary to ensure complete resolution (1, 2). Unfortunately, the different antifungal drugs in the current medical armamentarium all have shortcomings when used for a prolonged period of time (3). For invasive aspergillosis (IA), voriconazole became the first-choice treatment after an improved survival was documented over conventional amphotericin B (cAmB). Furthermore, voriconazole has a favourable adverse event profile compared to conventional formulations of amphotericin B and it is rarely associated with renal toxicity (4, 5). Nonetheless, no direct comparison has been made between voriconazole and liposomal amphotericin B (LAmB), which is a more well-tolerated form of amphotericin B than cAmB. In recent years, increasing rates of triazole resistant *Aspergillus* fumigatus in particular in Europe but also in other continents have become a major concern (6-10). This has led to a renewed incentive to reconsider therapeutic strategies using LAmB (11, 12). For many IFI caused by non-*Aspergillus* fungi, e.g. *Mucorales* spp., LAmB already is the preferred first-line treatment (13, 14). Therefore, treatment with LAmB is increasingly indicated and sometimes even the last resort in the management of IFI.

LAmB is solely administered in an intravenous formulation. Both safety concerns and logistical reasons prevent dismission from the hospital during intravenous treatment; however, often the treatment duration is long and exceeds the period of necessity of hospitalisation for other clinical reasons (1, 2). The practical limitations of daily intravenous treatment are evident. Reduction of duration of hospital stay would be favourable when considering both patient quality of life as well as economic costs. Furthermore, continued daily intravenous administration will lead to high cumulative dosages, associated with a higher rate of adverse events.

As an alternative, we have started to apply Outpatient Antifungal Therapy (OPAT) with LAmB. OPAT has been implemented successfully in the past with various antibiotics. In bacterial infections, increasing antimicrobial resistance rates have made prolonged intravenous treatment with reserve antibiotics necessary. For example, the increasing rate of Methicillin Resistant *Staphylococcus aureus* has been an important reason to apply prolonged OPAT with vancomycin (15-17). With LAmB, outpatient use has recently been implemented in a prophylactic setting (18).

Two recent reviews of the pharmacokinetic properties of LAmB strengthen the hypothesis that LAmB can effectively be applied as OPAT (19, 20). LAmB has a relatively short elimination half-life of 7 hours shortly after initiation of therapy, which increases to over 100 hours after prolonged use. This phenomenon is attributed to accumulation in tissues and slow redistribution (21, 22). When these pharmacokinetic properties of LAmB are taken into account (23-25), it can be expected that a therapeutic concentration can be attained using a less

frequent dosing scheme. Moreover, it may be possible to (partially) avoid nephrotoxicity if the total dose of LAmB is spread over multiple days (25, 26). Nephrotoxicity however remains an important caveat in the application of OPAT with LAmB, as mentioned in pharmacological review papers and in previous experimental experience (19, 20, 22, 27).

For those in need of prolonged antifungal treatment, step-down therapy to intermittent dosing in the context of outpatient treatment could offer similar efficacy with the potential of improved safety. An intermittent dosing strategy is occasionally applied in several hospitals in the Netherlands and Belgium. In this study, we are introducing the concept of treatment of IFI with intermittent LAmB dosing as OPAT.

METHODS

Study setting and patient population

A multi-centre retrospective cohort study was conducted within the Netherlands and Belgium. Hospitals that participate in the Dutch-Belgian Mycoses study group (DB-MSG) (28), a consortium committed to the clinical research of IFI, were sent an inquiry about their experience in the application of OPAT with LAmB in the past 10 years. Of the 11 medical centres that participate in the DB-MSG, four responded that they had applied OPAT with LAmB in recent years. OPAT was applied at the home of the patient or within the hospital outpatient department. All adult patients treated with LAmB with a less frequently than daily administration via the outpatient departments of Leiden University Medical Center, Erasmus MC Rotterdam, Radboud University Medical Center Nijmegen, and the University Hospitals Leuven since January 2012 were included. These centres are all tertiary care university hospitals and engaged in extensive solid organ and haematopoietic stem cell transplantation programs.

Study protocols and definitions

No uniform protocols for the start of intermittent therapy with LAmB were present. Typically for *Aspergillus* disease, a 3 mg/kg dose was started. For *Mucor* species a typical dose was between 5-10 mg/kg. The choice to start treatment with intermittent therapy with LAmB was made according to the clinical judgement of the treating physician usually based on imaging and the clinical course. Patients that were started on OPAT with LAmB were closely monitored for the occurrence of nephrotoxicity and most patients received the drugs in the outpatient department of the hospital. In the first month, all patients had at least a weekly monitoring of electrolyte and kidney function. In the subsequent weeks, monitoring occurred at least once every two weeks.

Nephrotoxicity was defined as a >1.5 times increase of baseline serum creatinine levels resulting in an eGFR of less than 40 ml/min/1.73 m^2 during treatment or as electrolyte disorders suspected to be the result of renal damage and requiring cessation of treatment with LAmB at

the discretion of the treating physician. Resolution of IFI was defined as clinically observed absence of symptoms that are likely to be caused by IFI in combination with findings concordant with resolution of IFI on high-resolution CT-scan and the absence of the need to restart antifungal therapy within 6 months.

Data collection

At the participating sites, lists of patients that received LAmB as an outpatient were provided by the hospital pharmacy. Based on these lists, the electronic medical records were examined to ensure eligibility for inclusion in our study. The only inclusion criterion was at least 2 weeks of intermittent treatment outside of the hospital with LAmB for an IFImeeting the diagnostic criteria of the 2008 revised EORTC/MSG definitions for invasive fungal infections (29).

After retrieval of all relevant information, the data of all participants were pseudonymized. Patient characteristics including age, diagnosis of immunocompromising disease, diagnosis of IFI, comorbidity and immune status were collected, as well as information about performed diagnostics, dosage of therapy, duration of therapy, treatment history, switch of antifungal therapy, renal function and outcome of the IFI. The latter three variables were considered the primary study outcomes to assess the safety and efficacy of this strategy. IFI were classified according to the 2008 revised European Organisation for Research and Treatment of Cancer – Mycoses Study Group criteria for the classification of IFI (29).

Analyses

Descriptive statistics of clinical variables of patients were calculated using the complete dataset. Kaplan Meier curves of survival during OPAT with LAmB were constructed. The analyses were performed using STATA v 16 (Statacorp, College Station, Texas, USA).

Ethics

The study was reviewed by the institutional review board of the LUMC Leiden in the Netherlands, which confirmed that the study did not fall under the Dutch law on research on human subjects. The institutional review board from UZ/KU Leuven in Belgium approved the study. Data were processed after pseudonymization by the local investigators and in accordance with Personal Data Protection Acts of the respective countries.

RESULTS

Between January 1st 2010 and September 1st 2018, a total of 18 adult patients received LAmB as an outpatient in a dosing frequency of two or three times a week. Triazole resistance, demonstrated by either PCR or culture, has been the most common reason (in 10 cases) to choose treatment with LAmB instead of voriconazole in the patients with IA. Of all patients,

nine (50%) were male and median age was 60 years. Fourteen patients (78%) had a haematological malignancy as underlying predisposing disease. Other underlying diseases were chronic obstructive pulmonary disease (COPD), sickle cell disease and chronic granulomatous disease(CGD). Suspected causative agents of IFI were *Aspergillus* spp. (12 patients), *Mucorales* spp. (3 patients), *Fusarium* spp. (2 patients) and a combination of both *Aspergillus* and *Mucor* (1 patient). Table 1 summarises the descriptive characteristics of the study cohort. A response to treatment was confirmed by CT-scan in 17 patients prior to discharge and start of OPAT with LAmB. For the remaining patient, clinical improvement had been the reason to proceed with OPAT. Patients switched from daily treatment as an inpatient to intermittent OPAT with LAmB after a median of 56 days (range 14-193 days). Median dosage of liposomal amphotericin B was 3 mg/kg, administered three times each week. Some patients switched drug dosage and/or frequency as detailed in the legend. None of the patients received combination therapy. Resolution of infection was finally achieved in 13 patients. The remaining patients were readmitted to the hospital, switched to another antifungal, died or were lost to follow-up.

Table 1: Patient characteristics

Total number of patients	18
Patient Characteristics	
Sex, male (%)	9 (50)
Age, median (range)	60 (18-78)
Underlying predisposing disease, number of pts. (%)	
ALL	6 (33)
AML/MDS-RAEB2	4 (22)
CLL	3 (17)
COPD	2 (11)
Aplastic anemia	1 (6)
CGD	1 (6)
Sickle cell disease	1 (6)
Prior allogeneic HSCT for any underlying disease	8 (44)
Invasive fungal infection, number of pts. (%)*	
Aspergillosis	13 (72)
Mucormycosis	3 (17)
Fusariosis	2 (11)
Cryptococcosis	1 (6)
Reason to treat invasive aspergillosis with LAmB, Number of patients (% of patients with IA)	
Triazole resistance identified with culture or PCR	10 (77)
Resistance presumed because IA occurred despite adequate prophylaxis with a triazole	2 (15)
Resistance presumed because IA showed progression despite adequate treatment with a triazole	1 (8)

Table 1: Patient characteristics (continued)

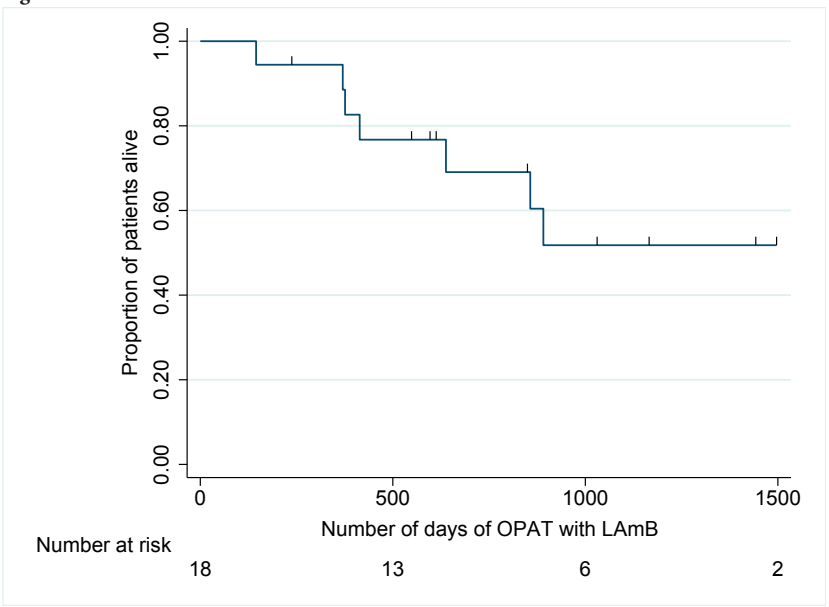
Treatment	
Dosage in mg/kg and frequency in times/week†, number of patients treated with the regimen at any point	
2 mg/kg 3 times/week	1
3 mg/kg 2 times/week	1
3 mg/kg 3 times/week	12
5 mg/kg 3 times/week	2
6 mg/kg 3 times/week	5
10 mg/kg 2 times/week	2
Response to treatment confirmed by CT before start of intermittent therapy, number of pts (%)	17 (94)
Number of days between date of diagnosis and start of intermittent therapy, median number of days (range)	56 (14-193)
Nephrotoxicity^, number of patients (%)	
Occurrence of nephrotoxicity at some point during intermittent LAmB treatment Of which	10 (56)
- resulting in switch to other antifungal agent	1 (10)
- resulting in cessation of antifungal treatment (because of concurrent sufficient clinical and radiological response to treatment)	4 (40)
- resulting in dose or frequency reduction~	5 (50)

Legend: LAmB denotes liposomal amphotericin B, ALL acute lymphoid leukaemia, AML acute myeloid leukaemia, MDS-RAEB2 myelodysplastic syndrome - refractory anaemia with excess blasts-2, CLL chronic lymphatic leukaemia, HSCT haematopoietic stem cell transplantation, CT computed tomography, COPD chronic obstructive pulmonary disease, PCR polymerase chain reaction, CGD chronic granulomatous disease, IA invasive aspergillosis. * Numbers add up to more than 100% due to one patient suffering from an infection caused by both *Mucor* and *Aspergillus*. ^Nephrotoxicity defined as either serious electrolyte disturbances necessitating treatment cessation at the discretion of the treating clinician or at least 50% increase of creatinine levels resulting in a eGFR of less than 40 ml/min. †Numbers add up to more than 100% because of 5 patients with dose alterations during the study period. ~ Dose reductions were as follows: 2 patients treated with 6 mg/kg 3 times weekly and 1 patient treated with 5 mg/kg 3 times/week were switched to 3 mg/kg 3 times weekly. Of 2 patients treated with 3mg/kg 3 times/week, one was switched to 3mg/kg 2 times/week and 1 patient was switched to 2mg/ kg 3 times/week. Kidney function normalised in all 5 patients. ‡ Resolution of infection defined as clinically observed absence of symptoms that are likely to be caused by invasive fungal infection in combination with clinically irrelevant or absent abnormalities concordant with invasive fungal infection on high-resolution CT-scan.

Nephrotoxicity during OPAT occurred in 10 cases, of which in only one case treatment needed to be switched to another antifungal agent (posaconazole, after determining intermediate sensitivity).

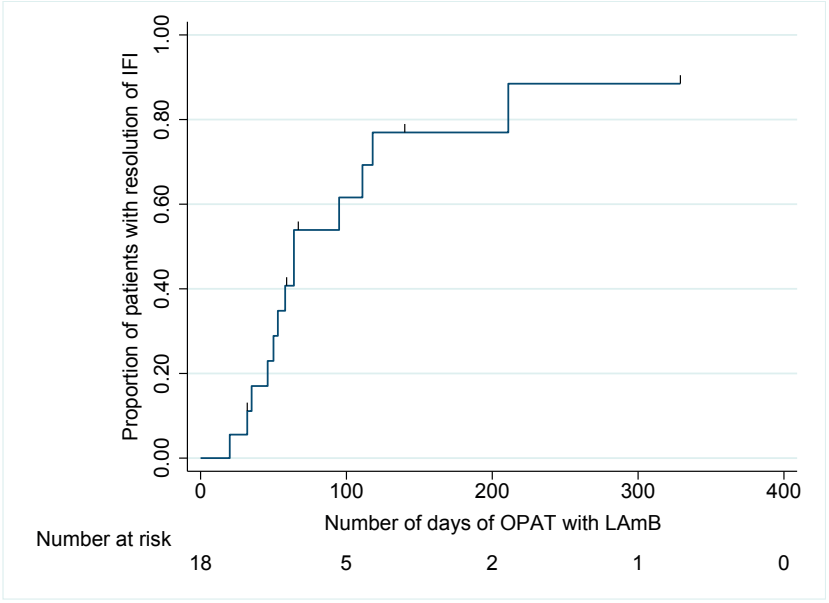
All patients in our dataset had normalised renal functions after decreasing of dosage or cessation of LAmB therapy. Severe hypokalaemia (less than 2.5 mmol/litre) was not observed during treatment with LAmB in an intermittent scheme. Oral substitution of potassium had been applied in 2 cases. Potassium levels raised to normal during treatment in one of these patients, and soon after the end of intermittent treatment in the other patient.

Figure 1A: Overall survival from start of intermittent treatment



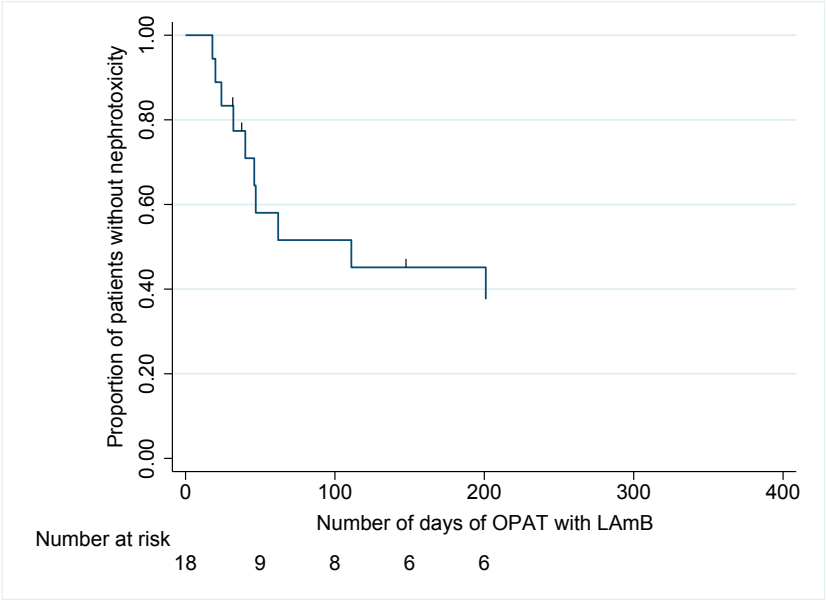
Legend: OPAT denotes outpatient parenteral antifungal therapy, LAmB liposomal amphotericin B. Censored cases were lost to follow up.

Figure 1B: Time to resolution of IFI after start of intermittent therapy.



Legend: IFI denotes invasive fungal infection, OPAT outpatient parenteral antifungal therapy, LAmB liposomal amphotericin B. Censored cases stopped intermittent treatment before resolution of infection. Resolution of IFI was defined as clinically observed absence of symptoms that are likely to be caused by IFI in combination with findings concordant with resolution of IFI on high-resolution CT-scan.

Figure 1C: Occurrence of nephrotoxicity from start of intermittent treatment



Legend: OPAT denotes outpatient parenteral antifungal therapy, LAmB liposomal amphotericin B. Censored cases stopped intermittent treatment before nephrotoxicity occurred. Nephrotoxicity was defined as a >1.5 times increase of baseline serum creatinine levels resulting in an eGFR of less than 40 ml/min/1.73 m^2 during treatment or as electrolyte disorders suspected to be the result of renal damage and requiring cessation of treatment with LAmB at the discretion of the treating clinician.

For the remaining cases, the treating physician opted for a dose reduction (four cases) or, after establishing a sufficient treatment response, for the cessation of antifungal therapy (five cases). The 100-day mortality and 1-year mortality were 0 and 1 patients out of 18 respectively. All-cause mortality until the end of follow-up was 39% but was primarily related to the underlying immunocompromising disease. In all cases treated for IA, the reason to treat with LAmB was triazole resistance (demonstrated in 10 patients, presumed in 3 patients). Readmission to the hospital was necessary due to factors related to the infection (3 patients) or to LAmB-related nephrotoxicity (1 patient). Figure 1a shows the survival rates of all patients in a Kaplan Meier analysis since start of OPAT. Figure 1b shows the time until resolution of infection. Figure 1c shows the time until nephrotoxicity occurred during intermittent treatment.

Table 2: Outcomes

Outcome	N=18
Median number of days of follow-up, median (range)	741 (145-2543)
All-cause mortality at end of follow-up, number of pts (%)	7 (39)
100 day mortality after start of OPAT, number of pts (%)	0 (0)
1 year mortality after start of OPAT number of pts (%)	1 (6)
Resolution of infection‡, number of pts (%)	13 (72)

Legend: ‡ Resolution of infection defined as clinically observed absence of symptoms that are likely to be caused by invasive fungal infection in combination with clinically irrelevant or absent abnormalities concordant with invasive fungal infection on high-resolution CT-scan

DISCUSSION

This study shows that the use of OPAT with LAmB in a 2 or 3 times weekly dosing scheme results in high rates of therapy response in a selected patient population and after confirmation of an initial response to daily IV therapy with LAmB. However, safety issues did arise, resulting in mostly reversible nephrotoxicity and in some cases infection or therapy-related readmission to the hospital.

The majority of patients in this study needed prolonged use of LAmB for the treatment of triazole resistant *A. fumigatus* infections. After the first reports of voriconazole resistant A. fumigatus appeared in 2009 from the Netherlands (30), triazole resistance has now extensively been reported in many regions all over the world (7, 11). Although the prevalence is low in some regions, the rates have been steadily increasing in others (7, 31). The high rates of triazole resistance also impact decision making in patients for whom susceptibility testing is not possible. In many cases, the clinician may fear presence of resistance in case of worsening of clinical or diagnostic parameters after treatment with a triazole even with negative or absent resistance tests. Because of difficulty in establishing triazole-resistance or sensitivity, the clinical suspicion of resistance is becoming an important reason to abstain from further treatment with triazoles and opting for LAmB instead. Fortunately, more possibilities to detect resistance have become available. The impact of resistance testing of IA using PCR is expected to more effectively guide the clinician in the optimal choice of therapy (32) and is being evaluated in a prospective multicentre study in the Netherlands and Belgium (NCT03121235).

Renal toxicity

Since the introduction of (conventional) amphotericin B as treatment of fungal infections, nephrotoxicity has been a major concern. Nevertheless, nephrotoxicity has significantly decreased after the introduction of the liposomal formulation of amphotericin B (33-36). In particular, patients that need prolonged therapy and are exposed to high doses over a prolonged period of time are vulnerable for the development of renal adverse events. A decrease in dosage

could also be beneficial in mitigating the drug-related renal toxicity. However, nephrotoxicity occurring at the end of the anticipated therapy period has been a reason to stop antifungal treatment prematurely and instead evaluate the natural course of the disease. Importantly, the associated nephrotoxicity was reversible in the majority of cases after cessation of therapy or dose alteration. The occurrence and time course of nephrotoxicity did differ from literature describing patients with daily dosing (37-39). Additionally, some experience in the assessment of the safety of the use of LAmB in an outpatient setting is previously described by Malani et al in 2005 (27). The authors of this study also found high rates of nephrotoxicity; the results are nonetheless not directly comparable due to their inclusion of application of non-lipid formulations of amphotericin B. The mentioned literature reports generally lower rates of reversibility of nephrotoxicity and shorter duration until occurrence of nephrotoxicity. However, a recent study also reports a high rate of reversibility of nephrotoxicity after use of LAmB (40). Possibly, our data supports the theory that nephrotoxicity occurs later and has a higher probability to be reversible when applying LAmB in an intermittent dosing schedule.

Application of OPAT strategies are slowly expanding within the field of infectious diseases and are being implemented in regular practice. Similar to LAmB, intravenous vancomycin therapy is also associated with renal toxicity but has nonetheless been successfully implemented in an OPAT programme for many years now (16, 17). Despite early reluctance, the expected logistic and toxicity-related disadvantages (41, 42) are outweighed by the advantages of a decrease in hospital stay with similar therapeutic effectiveness thanks to the implementation of monitoring of toxicity and therapeutic drug monitoring (15, 17, 43).

Study strengths and limitations

Despite a nation-wide inquiry, only a small subset of adult patients treated for IFI have been identified. The means by which these patients have been selected to undergo OPAT is inherently biased, i.e. the decision of the clinician to apply this therapeutic strategy has been dependent on many confounding factors, both known and unknown. Since no guideline refers to or advises OPAT with LAmB, and due to lack of supportive literature, physicians may only have elected this approach in specific situations. Additionally, lack of existing intra- or extramural infrastructure to apply OPAT could be a limiting factor. Due to this selection, presumably patients with a relatively favourable prognosis with regard to the IFI were included in our study. Also, the heterogeneity of both the patient population and the different dosings that have been used make it difficult to draw any solid conclusions about efficacy and tolerability. As it is impossible to adjust for all of these factors, the results of our study cannot be directly compared with other cohorts of patients with IFI. However, the baseline variables that have been presented, summarise the most important characteristics, possibly contributing to identifying potentially eligible patients for this treatment strategy. Only patients with an initial response to therapy with LAmB showing no or only mild prior adverse events related to LAmB use were subjected to this strategy. Hence, the involved physicians balanced the risks of

inadequate treatment of IFI against the advantages of treatment in the outpatient setting. For future adaptation of this strategy, it is important for the clinician to weigh these factors before deciding on applying OPAT with LAmB.

Summary and conclusions

After documentation of an initial treatment response and in a selected patient group, intermittent therapy with LAmB in the outpatient setting appeared to be a valuable treatment option for IFI. Frequent monitoring of renal function and potassium levels, for example once every week, is strongly recommended for early recognition of nephrotoxicity, as it can also occur during prolonged OPAT. This treatment strategy is expected to provide advantages in costs, decrease of hospital-associated infections and patient's quality of life. Further research will be necessary to expand upon the possibilities that this treatment strategy offers. The identification of eligible patient populations that would most benefit from this strategy as well as further study of the toxicity concerns in this setting are warranted.

Contributions: RP, AS and RD performed the data collection. RP wrote the first draft of the manuscript. RP, JW and MB were involved in the concept and design of the study. MB, BR, RB and IS acted as local main investigators in their respective centres and provided the data. Analyses were performed by RP in collaboration with MB. All authors critically revised all drafts of the manuscripts and approved the final version.

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Chapter 7

Implementation of a clinical decision rule for selecting empiric treatment for invasive aspergillosis in a setting with high triazole resistance.

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ABSTRACT

Objectives: World-wide, emerging triazole resistance increasingly complicates treatment of invasive aspergillosis (IA). In settings with substantial (>10%) prevalence of triazole resistance, empiric combination therapy with both a triazole and liposomal amphotericin B (LAmB) can be considered because of the low yields of susceptibility testing. To avoid toxicity while optimizing outcome, a strategy with monotherapy would be preferable.

Methods: A newly designed treatment algorithm based on literature and expert consensus provided guidance for empiric monotherapy with either voriconazole or LAmB. Over a four and a half year period, all adult patients treated for IA were included and patient data were collected. An independent committee reviewed the attributability of death to IA for each patient. Primary outcomes were 30- and 100-day crude mortality and attributable mortality.

Results: In total, 110 patients were treated according to the treatment algorithm. Fifty-six patients (51%) were initially treated with voriconazole and 54 patients (49%) with LAmB. Combined attributable and contributable mortality was 13% within 30 days and 20% within 100 days. Treatment switch to LAmB was made in 24/56 (43%) of patients who were initially treated with voriconazole. Combined contributable and attributable 100-day mortality in this subgroup was 21% and was not increased when compared with patients initially treated with LAmB (p=0.38).

Conclusions: By applying a comprehensive clinical decision algorithm, an antifungal-sparing regime was successfully introduced. Further research is warranted to explore antifungal treatment strategies that account for triazole-resistance.

INTRODUCTION

Since the introduction of the antifungal drug voriconazole, triazole formulations are the primary treatment of choice for invasive aspergillosis (IA) in leading European and American guidelines (1- 3). However, over the past decades, emerging triazole resistance has developed as a new important threat to effective prevention and treatment of IA (4-7). This development has given rise to an increase in the application of liposomal amphotericin B (LAmB), which is the drug of second choice for this indication (8, 9). This is concerning due to decreased efficacy and increased toxicity of LAmB when compared to voriconazole (1, 10-17). The highest incidence rates of IA can be found in patients with a haematological malignancy who receive intensive chemotherapeutic treatment and/or undergo haematopoietic stem cell transplantation (HSCT) (18-21). Despite the use of antifungal chemoprophylaxis, the residual incidences observed in this population often remain 5-10% or more (21-23). Furthermore, due to the increasing number of patients that survive with temporary or chronic diseases of the immune system and by introduction of new treatment modalities e.g. tyrosine kinase inhibitors like ibrutinib, the population at risk of IA is expanding (15, 24, 25).

Mutations in the fungal DNA that are responsible for inefficacy of voriconazole, are most often associated with pan-triazole resistance and therefore necessitate the use of LAmB. Use of LAmB is associated with lower rates of treatment success and high rates of toxicity when compared to voriconazole (10, 12, 13). Demonstrated resistance against all triazoles is a clear indication for the use of LAmB (9). Deciding between voriconazole and LAmB as empiric therapy is difficult when the clinician suspects the presence of triazole resistant IA, but definite proof is missing. The background resistance rate, which varies among different populations at risk and different regions, needs to be taken into account when making this decision.

The first clinical isolates with triazole-resistance mechanisms have been recognised in Northwestern Europe (7, 26). At present, resistance against triazoles in environmental as well as clinical isolates has developed to a global problem (27-32). Results of a nationwide surveillance study in 2014 showed a triazole resistance rate of 5% in the USA, and presence of resistant isolates in the environment has been demonstrated as well (29, 31). In the Netherlands, average resistance rates of *Aspergillus fumigatus* were 14.7 % in academic hospitals and 7.8% in non-academic teaching hospitals in 2018 (42). It is complicated to measure the impact of triazole resistance in the clinical setting. Due to the fact that cultures and antifungal susceptibility testing fails in the majority of clinical specimens, the clinician often has to resort to an empirical treatment strategy. Although our ability to determine susceptibility has recently been improved due to the introduction of PCR, the combined results of both culture and PCR are conclusive only in 30-60% of patients with probable IA (33-35). The resulting uncertainty about susceptibility easily gives rise to overtreatment with LAmB. When there is a high background resistance rate, any clue that raises the suspicion of resistance could motivate the clinician to opt for the use of LAmB instead of voriconazole. To ensure both the addition of the survival benefit of treatment

with voriconazole and covering the risk of triazole resistance, Dutch national guidelines advise to empirically treat IA with combination therapy of both voriconazole and LAmB in case of unknown susceptibility (36). However, the expected benefits of this strategy need to be weighed against a higher rate of serious adverse events associated with combination therapy, as well as higher costs (16, 37, 38), but no randomised study data are available on this topic. Furthermore, no randomised head-to-head comparison between LAmB and voriconazole has been published, leaving some uncertainty about the superiority of voriconazole (10-13).

To evade unnecessary toxicity while optimizing outcome, a clinical decision rule guiding to monotherapy with either voriconazole or LAmB was designed and validated in our hospital in a region with resistance rates between 16 and 24 percent.

METHODS

Development of a clinical treatment strategy

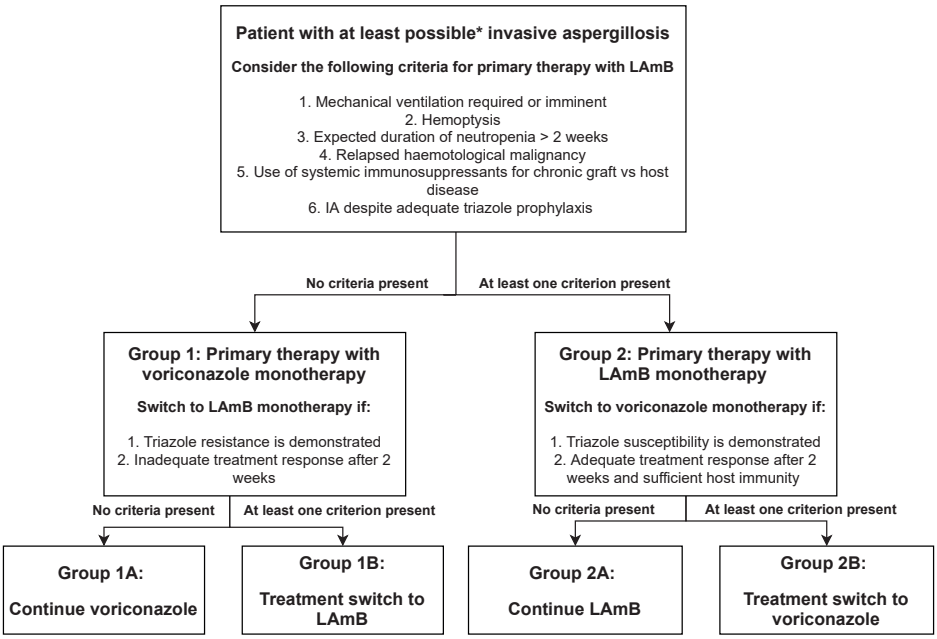
In 2014, all medical specialties in our center involved in the diagnosis and treatment of patients with IA participated in constructing a treatment algorithm that provided guidance for empiric monotherapy with either voriconazole or LAmB (figure 1). The algorithm aimed to optimise the balance between the risk of treating triazole-resistant IA with voriconazole and the risk of unnecessarily treating triazole-susceptible IA with LAmB. The treatment protocol was based on literature and guidelines (1, 21, 23, 37, 39-41) and information about local resistance rates (42).

Criteria were formulated that either predispose for a worse outcome (e.g. presence of respiratory insufficiency) or were thought to predispose for infection with a triazole-resistant isolate (development of IA during chemoprophylaxis with a triazole). Both predicted worse outcome and breakthrough infections were considered indications for empiric treatment with LAmB. Although it is currently not supported by the literature that prophylaxis using a triazole predisposes for infection with a triazole-resistant isolate, the committee that constructed the algorithm decided to apply this criterion based on its rational concept. The reason to initially treat patients with a predicted worse outcome with LAmB was to mitigate the risk of applying ineffective treatment to a severely ill patient with triazole-resistant IA. Combination therapy was not applied to avoid possible toxicity or other complications. The designed algorithm was approved by the institution's antimicrobial steering committee and was implemented from January 2015 onwards.

Study protocols and definitions

All patients who were considered to have a high risk of IA routinely received prophylaxis with a triazole with activity against *Aspergillus*. This included patients receiving remission-induction therapy prior to allogeneic HSCT, patient who underwent allogeneic HSCT, patients with prolonged neutropenia (at least two weeks), or other patients receiving high risk chemothera-

Figure 1: Flowchart of the treatment protocol



Legend: IA denotes invasive aspergillosis; LAmB liposomal amphotericin B. *As defined by the 2008 EORTC/MSG criteria for the diagnosis of invasive fungal infections (39)

peutics that induce neutropenia or impaired granulocyte function. Therapeutic drug monitoring was applied in this population (43).

Protocolised CT-scanning and bronchoalveolar lavage (BAL) were performed upon suspicion of IA. BAL samples were examined by direct microscopy, culture, galactomannan assay (cut off at 0.5 optical density) and from 2017 onwards also by PCR. All available BAL samples from patients not tested by PCR were retrospectively tested. Triazole resistance was routinely tested by four well agar plate screening (VIP check, Groningen, The Netherlands).

Data collection

All adult patients were retrospectively included if they had been treated according to our treatment protocol between January 2015 and September 2019. Patients who received either LAmB, voriconazole, isavuconazole or posaconazole within the Leiden University Medical Center were identified through the hospital pharmacy database. Of these patients, the electronic medical records were examined to ensure eligibility for inclusion in our study. Extracted patient characteristics included age, diagnosis of immunocompromising disease, diagnosis of IA, comorbidity and immune status, as well as information about performed diagnostics, triazole susceptibility, dosage of therapy, duration of therapy, treatment history, switch of antifungal therapy, renal function and outcome of IA. IA was classified according to the 2008

revised European Organisation for Research and Treatment of Cancer – Mycoses Study Group criteria for the classification of invasive fungal infections (39). After retrieval of all relevant information, the data of all participants was pseudonymised.

Definition of attributable mortality

The role of IA with regard to the cause of death was classified as either 'non-attributable to IA', 'contributable', 'attributable', or 'unknown' (see criteria in the **text box**). The last category was introduced because the clinical data at time of death were insufficient for a few cases. Attributable and contributable mortality were the primary outcome measures for this study. The classification was constructed by the investigator group prior to analysis of the data and was based on modification of definitions from literature (40, 44). A committee was instructed to determine attributability in all patients who died within 100 days. The committee consisted of three independent reviewers involved in the fields of infectious diseases, clinical microbiology and haematology. The reviewers received written instructions to use the medical correspondence, post-mortem reports and laboratory findings as reported in the patient files to categorise the deceased patients according to the above definitions. In case of disagreement between the reviewers, the case was discussed with all three reviewers until consensus was reached.

Statistics

Survival proportions were calculated and comparative analyses were performed using SPSS version 25 (IBM Corp Armonk, NY). Fisher's exact test was used to calculate p-values.

Ethics

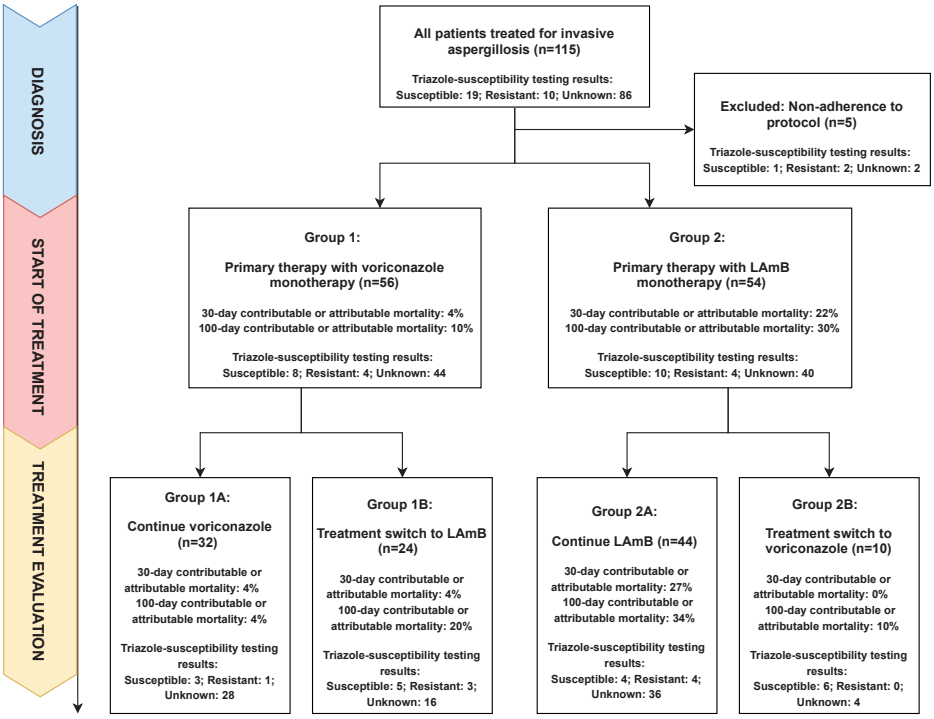
The study was reviewed by the institutional review board, which deemed that the Dutch law on research on human subjects was not applicable to our study. Data were processed in accordance with the national Personal Data Protection Act.

RESULTS

Protocol adherence and study population characteristics

A total number of 115 patients were treated for IA at our institution from January 2015 to September 2019. Of this total, 110 patients (96%) were treated for IA with either voriconazole or LAmB monotherapy according to the designed algorithm. The remaining five patients received off-protocol treatment regimens (figure 2). These patients received either combination therapy, or violated the criteria mentioned in the decision tool otherwise. For all results, the remaining 110 patients were used to calculate percentages, with the exception of the triazole resistance rates.

Figure 2: The treatment protocol with numbers of patients in each arm as well as resistance and mortality rates.



Legend: LAmB denotes liposomal amphotericin B. Susceptibility testing was done by applying both PCR and culture techniques. Contributable and attributable mortality rates were defined in the process as described in the methods section and can also be found in table 3. *Possible invasive aspergillosis was defined according to the 2008 revised definitions for the diagnosis of invasive fungal disease (39)

Study population characteristics

Out of 110 included patients, 76 (69%) were male; the median age was 63 (range 20-83), and 100 (90%) patients were treated for an underlying haematologic malignancy or had undergone allogeneic stem cell transplantation (table 1). Of patients with haematologic malignancy, 14 suffered from a relapsed malignancy and eight from a secondary (treatment related) malignancy. A small subgroup of patients (10%) did not suffer from haematologic malignancy but were recipients of a solid organ transplantation, received chemotherapy for other malignancies, or were immunocompromised for other reasons.

Treatment and outcomes

Fifty-six patients (51%) were initially treated with voriconazole and 54 (49%) were initially treated with LAmB. Cultures were positive in 16/115 (14%) patients and phenotypical voriconazole resistance was detected in 5/16 (31%). Overall, susceptibility testing was successful in 29 cases, yielding 10 (34%) triazole-resistant and 19 (66%) triazole susceptible isolates.

Table 1: Characteristics of patients treated for invasive aspergillosis

Total number of patients	110
Sex, male (%)	76 (69)
Age, median (range)	63 (20-83)
Underlying predisposing disease, number of pts. (%)	
Total with haematologic malignancy or after HSCT	100 (91)
Total who underwent HSCT	54 (49)
Patients with haematologic malignancy:	45 (41)
AML/MDS-RAEB2	29 (26)
ALL	12 (11)
Multiple Myeloma	4 (4)
MDS	4 (4)
Aplastic Anemia	2 (2)
CLL	2 (2)
Myelofibrosis	1 (1)
CML	
Received HSCT for sickle cell disease	
Receiving chemotherapy for solid tumor	2 (2)
Kidney transplantation	3 (3)
Liver transplantation	2 (2)
Other* (not malignant, not transplant-related)	3 (3)

Legend: IA denotes invasive aspergillosis, HSCT haematopoietic stem cell transplantation, LAmB liposomal amphotericin B, ALL acute lymphoid leukaemia, AML acute myeloid leukemia, CML chronic lymphoid leukemia, CLL chronic lymphoid leukemia, MDS myelodysplastic syndrome, MDS-RAEB2 myelodysplastic syndrome - refractory anaemia with excess blasts grade 2. * Other underlying diseases: severe anorexia nervosa, badly regulated diabetes type 1, and influenza.

Susceptibility was determined phenotypically by culture in 13 clinical isolates, and genetically with PCR in 16 clinical isolates. In one case, triazole-resistance was detected by both PCR and culture.

Upon clinical evaluation of empiric therapy and of resistance data, a switch was made to LAmB in 24/56 (43%) of patients who were initially treated with voriconazole. Table 2 displays the reasons for treatment switch. In the group that started treatment with LAmB, 9 out of 54 (17%) patients switched to voriconazole. Treatment was completed with voriconazole in 42 cases and with LAmB in 68 patients. The flowchart (figure 2) shows the number of patients in each treatment group.

Therapy-related adverse events occurred in both treatment arms. Hepatotoxicity was a reason to stop treatment with voriconazole in seven patients and nephrotoxicity was a reason to stop treatment with LAmB in 11 patients. Allergic reaction was a reason to stop treatment with voriconazole in one case. Serious alteration of mental state and/or visual hallucinations were a reason to stop treatment with voriconazole in two cases. Reasons to start treatment with

Table 2: Motivation of treatment decisions

Number of patients initially treated with voriconazole (group 1)	**56**
Number of patients initially treated with LAmB (group 2)	**54**
*Reason to initially treat with LAmB**, number of patients, (% of patients treated with LAmB)	
Mechanical ventilation required or imminent	9 (17)
Hemoptysis	0
Expected duration of neutropenia >2 weeks	2 (4)
Relapsed haematologic disease	14 (26)
Use of systemic immunosuppression for chronic graft versus host disease	0
IA occurred despite adequate prophylaxis with a triazole	28 (52)
Broader antifungal spectrum deemed necessary (e.g. suspicion of mucormycosis)	5 (9)
Intolerance or significant drug interaction with voriconazole	4 (7)
Number of patients who switched from voriconazole to LAmB (group 1B)	**24 (23)**
Reasons to switch, number of patients, (% of patients who switched)	
Resistance to azoles demonstrated	3 (13)
Progression of IA	15 (63)
Intolerance to voriconazole	6 (25)
Number of patients who switched from LAmB to voriconazole (group 2B)	**10 (9)**
Reasons to switch, number of patients (% of patients who switched)*:*	
Susceptibility to azoles demonstrated	6 (60)
Adequate treatment response and sufficient recovery of host immunity	2 (20)
Intolerance to LAmB	2 (20)

Legend: LAmB denotes liposomal amphotericin B, IA invasive aspergillosis. *More than one reason could be present for one patient

LAmB are displayed in table 2. The most common reason to initially treat with LAmB was adequate prophylaxis with voriconazole before establishing the diagnosis of IA (28 patients). Of these 28 patients, susceptibility testing was successful in four cases, of which only one was triazole-resistant.

Crude and attributable mortality rates per treatment stratum are listed in table 3. The flowchart (figure 2) shows a combination of attributable and contributable rates only. Contributable or attributable mortality was lower in patients initially treated with voriconazole (30-day contributable or attributable mortality was 6%) than patient initially treated with LAmB (30-day contributable or attributable mortality was 22%). Mortality was lowest in patients who were only treated with voriconazole monotherapy. Mortality was highest in patients who were only treated with LAmB monotherapy and in patients initially treated with voriconazole and later switched to treatment with LAmB as per the rules of the decision tree. When comparing these two groups, the mortality rates differed only slightly without statistical significance (61% vs 54% crude 100-day mortality, 34% vs 21% combined attributable and contributable 100-day mortality). Attributable mortality was highest in the first period after diagnosis of IA.

Table 3: Outcomes of patients treated according to the protocol

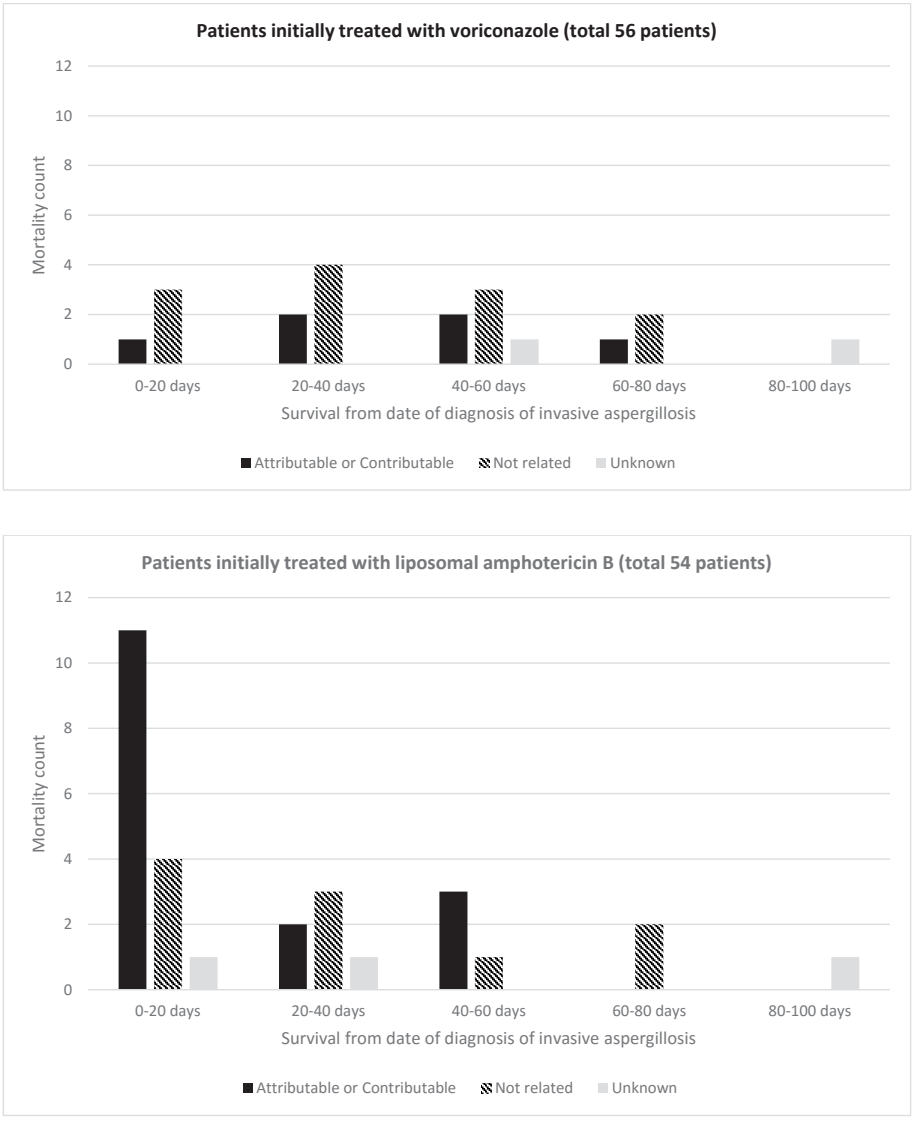
	Total	Group 1 (VOR)	Group 2 (LAmB)	Group 1A (VOR)	Group 1B (VOR, LAmB)	Group 2A (LAmB)	Group 2B (LAmB, VOR)
Number of patients	**110**	**56**	**54**	**32**	**24**	**44**	**10**
30-day mortality							
# patients (%)							
total	**26 (24)**	**7 (13)**	**19 (35)**	**4 (13)**	**3 (13)**	**19 (43)**	**0**
attributable	3 (3)	2 (4)*	1 (2)*	1 (4)	1 (4)	1 (2)	0
contributable	11 (10)	0*	11 (20)*	0	0	11 (25)	0
unrelated	11 (10)	5 (9)	6 (11)	3 (9)	2 (8)	6 (11)	0
unknown	1 (1)	0	1 (2)	0	0	1 (2)	0
100-day mortality							
# patients (%)							
total	**51 (46)**	**20 (34)**	**31 (57)**	**9 (28)**	**11 (54)**	**27 (61)**	**4 (40)**
attributable	6 (5)	3 (5)	3 (6)	1 (4)	2 (8)	2 (5)	1 (10)
contributable	16 (15)	3 (5)	13 (24)	0	3 (13)	13 (29)	0
unrelated	24 (22)	12 (21)	12 (22)	6 (18)	6 (28)	9 (20)	3 (30)
unknown	5 (9)	2 (4)	3 (6)	2 (6)	0	3 (7)	0
Azole-resistance ***# of patients (%)***	8 (5)	4 (7)	4 (7)	1 (3)	3 (13)	4 (10)	0
Azole-susceptibility ***# of patients (%)***	18 (15)	8 (14)	10 (19)	3 (9)	5 (21)	4 (9)	6 (60)
Diagnostic certainty ***# of patients (%)***							
Possible	36 (33)	16 (29)	20 (37)	13 (41)	3 (13)	19 (43)	1 (10)
Probable	72 (65)	39 (70)	33 (61	19 (59)	20 (83)	25 (57)	8 (80)
Proven	2 (2)	1 (2)	1 (2)	0	1 (4)	0	1 (10)

Legend: LAmB denotes liposomal amphotericin B, VOR voriconazole. Diagnostic certainty was defined according to the revised 2008 EORTC/MSG criteria for the diagnosis of invasive fungal infection (39). Group 1 consists of patient initially treated with voriconazole. Subgroup 1A continued treatment with voriconazole, while subgroup 1B switched to LAmB eventually. Group 2 consists of patients initially treated with LAmB. Subgroup 2A continued treatment with LAmB, while subgroup 2B switched to voriconazole eventually. The treatment rules for the different groups can be found in figure 1. *P-value = 0.002 of Fishers exact test for combined attributable and contributable mortality in group 1 versus group 2.

After 40 days, most mortality was either non-attributable to IA or of unknown cause (figure 3). Combined contributable and attributable mortality for all groups was 12% after 30 days and 20% after 100 days. Mortality unrelated to IA within 30 days was lower in patients with proven or probable IA only when compared to all patients (4% versus 10% overall).

The calculation of survival rates was repeated for proven and probable cases of IA (defined according to the 2008 revised EORTC criteria (39)) and detailed in supplement A. The results were similar to the results of the overall analysis.

Figure 3: Mortality counts of patients treated for invasive aspergillosis



Legend: IA denotes invasive aspergillosis. Attributable or contributable mortality was determined in the procedure as described in the methods section.

DISCUSSION

By applying a comprehensive clinical decision algorithm in our area with high (>10%) triazole-resistance rates, 51% of patients were empirically treated with monotherapy voriconazole,

without indications for excess crude mortality even if a later switch to LAmB was needed. In 29% of patients, therapy with LAmB could be avoided during the entire course of treatment. Our study provides a rationale to effectively account for possible triazole resistance while preventing the negative effects associated with combination therapy. However, due to the way the study has been designed, it is not possible to make direct comparisons between treatment groups; the basis on which the choices for therapy were made were dependent on clinical factors which are also associated with outcomes, thought to be helpful in identifying the patients who would benefit the most from the relevant treatment strategy. This distinction causes factors that are correlated with a worse outcome to not be equally distributed between the treatment groups. Mortality rates of patients initially directed to voriconazole monotherapy were lower when compared to people who were treated with LAmB. However, the group of patients that were initially treated with LAmB were expected to have a worse outcome at baseline.

The treatment decision tree was constructed in accordance with our local antibiotics steering committee, with knowledge about local epidemiology and triazole-resistance rates, current literature and the relevant guidelines. National and international guidelines that describe the optimal management for IA need to rely on low evidence levels, in part due to the relative rarity of IA. Additionally, because of improvements in the management of patients with haemato-oncological disease, the population at risk is becoming harder to define and is ever-changing. Emerging triazole resistance makes it even more difficult to formulate an unambiguous treatment advice.

No data are available that support an increased risk of triazole-resistant IA in patients that develop IA despite receiving adequate prophylaxis with a triazole. We did choose to include this as a criterion to initially treat with LAmB. Nonetheless, no remarkable additional risk of triazole-resistance was found in this subgroup (of four successful susceptibility tests in 28 patients, one isolate showed triazole resistance). A limitation of this study is that detection of resistance was not as successful as reported in literature despite the use of both PCR and conventional culture (33-35, 45). Reported rates of triazole resistant IA in our region are amongst the highest in the world, even within the Netherlands (32, 42). The high local resistance rates have been linked to the extensive use of fungicides in agriculture (7). All-cause mortality in our cohort was high with a 46% mortality rate within 100 days. In patients with proven and probable IA only, this rate was similar (41%). In literature, the mortality rates differed greatly between different subpopulations, but were on average lower than in our population (15, 22, 23). However, within the total mortality count, a minority of cases was attributable or contributable to IA. The nature of the subpopulation plays a large role in the expected case fatality rate. On average, our population consisted of patients with both a high risk of developing IA and a high risk of mortality due to haematological malignancy. Additionally, T-cell depleted haematopoietic stem cell transplantation is standard practice in our center. This method of transplantation is associated with a substantially lower risk of graft versus host disease, but at

the cost of more difficulty in the treatment of opportunistic infections due to an initially less effective T-cell mediated immune response.

In conclusion, the results of our study can provide new insight in the application of an antifungal-sparing clinical decision tool while minimizing the risks of the consequences of undertreatment. The non-randomised approach and heterogeneous population make it difficult to make generalised statements about treatment effectiveness, and future research could further expand on the hypothesis that LAmB can have an important role in the treatment of IA in areas with high triazole resistance without the necessity of combination therapy.

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Supplemental table A: Outcomes for patients with proven and probable invasive aspergillosis only

	Total	**Group 1 (VOR)**	**Group 2 (LAmB)**	**Group 1A (VOR)**	**Group 1B (VOR, LAmB)**	**Group 2A (LAmB)**	**Group 2B (LAmB, VOR)**
Number of patients	**74**	**40**	**34**	**19**	**21**	**25**	**9**
30-day mortality							
# patients (%)							
total	**12 (16)**	**3 (8)**	**9 (27)**	**1 (5)**	**2 (10)**	**9 (36)**	**0**
attributable	1 (1)	1 (3)*	0*	0	1 (5)	0	0
contributable	8 (11)	0*	8 (24)*	0	0	8 (32)	0
unrelated	3 (4)	2 (5)	1 (3)	1 (5)	1 (5)	1 (4)	0
unknown	0	0	0	0	0	0	0
100-day mortality							
# patients (%)							
total	**30 (41)**	**13 (33)**	**17 (50)**	**5 (26)**	**8 (38)**	**14 (56)**	**3 (33)**
attributable	4 (5)	2 (5)	2 (6)	0	2 (10)	1 (4)	1 (11)
contributable	11 (15)	2 (5)	9 (26)	0	2 (10)	9 (36)	0
unrelated	13 (18)	8 (20)	5 (15)	4 (21)	4 (19)	3 (12)	2 (22)
unknown	2 (3)	1 (3)	1 (3)	1 (5)	0	1 (4)	0
Azole-resistance *# of patients (%)*	8 (11)	4 (10)	4 (12)	1 (5)	3 (14)	4 (16)	0
Azole-susceptibility *# of patients (%)*	16 (22)	8 (20)	10 (29)	3 (16)	5 (23)	4 (16)	6 (66)
Diagnostic certainty *# of patients (%)*							
Probable	72 (97)	39 (98)	33 (97)	19 (100)	20 (95)	25 (100)	8 (89)
Proven	2 (3)	1 (2)	1 (3)	0	1 (5)	0	1 (11)

Legend: LAmB denotes liposomal amphotericin B, VOR voriconazole. Diagnostic certainty was defined according to the revised 2008 EORTC/MSG criteria for the diagnosis of invasive fungal infection (39). Group 1 consists of patient initially treated with voriconazole. Subgroup 1A continued treatment with voriconazole, while subgroup 1B switched to LAmB eventually. Group 2 consists of patients initially treated with LAmB. Subgroup 2A continued treatment with LAmB, while subgroup 2B switched to voriconazole eventually. The treatment rules for the different groups can be found in figure 1. *P-value < 0.001 of Fishers exact test for combined attributable and contributable mortality in group 1 versus group 2.

Chapter 8

Summary and general discussion

New developments and challenges in the management of invasive aspergillosis

In the last decades the field of invasive aspergillosis (IA) has been marked by four important developments. First of all, the population at risk for IA has increased due to the improvement in survival of the patients with a severely impaired immune system. New risk groups have been added to this population, such as patients who are treated with novel targeted therapies such as protein kinase inhibitors or patients with viral pneumonia caused by Influenza or COVID-19. Secondly, the development of new diagnostic techniques and the improvement of existing ones has led to increased accuracy regarding the diagnosis of IA. Thirdly, the development of new and effective triazoles and their implementation in the clinic, as well as the development of a safer and more effective formulation of amphotericin B, has resulted in improved survival and decreased toxicity in patients with IA. Finally, the emergence of triazole resistance of *Aspergillus* fungi has limited safe treatment options for IA. These four important developments have been presented graphically in the figure (figure 1). The different chapters in this thesis have all explored the impact of one or several of these developments for the patient with IA. In the following sections, all chapters are briefly summarized and the most important findings are discussed. In the last section, the topics are viewed in mutual interaction with each other and are put into a broader, future perspective.

Incidence and mortality of invasive aspergillosis.

All patient- or population-based interventions to minimize IA-related morbidity and mortality come with a certain cost. This cost can consist of toxicity of (prophylactic) antifungal drugs, unnecessary exposure to radiation from CT-scans or increased application of invasive diagnostics such as bronchoalveolar lavage (BAL). To properly assess the health gain of more aggressive prophylactic, diagnostic or therapeutic strategies targeted against IA, it is important to quantify the impact of IA in the population at risk. To attain this goal, first we need to know how many people are affected by IA. Secondly, we need to know the mortality resulting from IA.

To assess the incidence of disease, it is most important to identify the population at risk for IA. The size of this population has been rapidly expanding over the last decades. While the first cases of IA mostly occurred in patients with rare congenital immunodeficiencies or as a very unusual infection in patients with chronic lung disease, the population currently at high risk of IA mainly consists of patients with haematological malignancies. These patients are prone to IA due to alterations in the host defense against fungal pathogens by both the malignancy itself and, most importantly, by its treatment. The majority of clinical research concerning IA is conducted in this relatively well-described population at risk of IA.

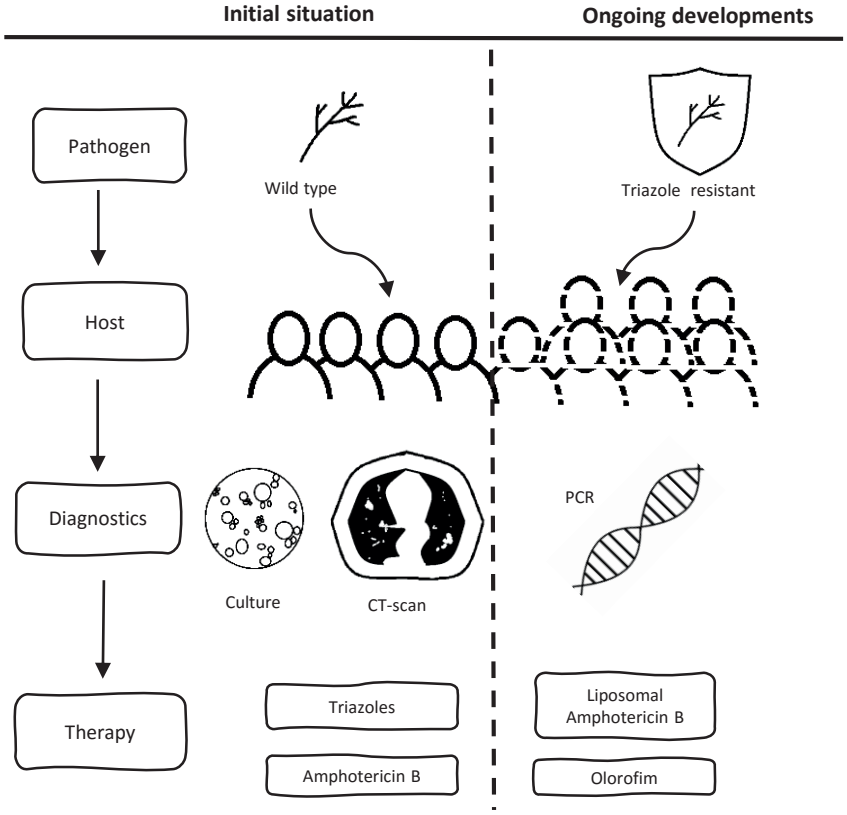
In chapter 2, we have analysed all research articles that report an incidence rate of IA. From data published in 49 articles, we have learned that on average 6% of all patients with a haematological malignancy have developed IA. Antifungal prophylaxis lowered the risk, but the incidence was still at least 4% in all subgroups. Antifungal prophylaxis only partly

protects against IA, and breakthrough infections remain an important problem. Patients who underwent an allogeneic haematopoietic stem cell transplantation had the highest risk (9%) of developing IA, despite receiving prophylaxis.

Mortality rates were high in all subgroups of patients, averaging about 29% mortality within 100 days. Variance in case fatality rates was very high, however.

Considering the prevalence of AML and other serious impairments of the immune system, IA has an important impact on health all over the world. Furthermore, the high rate of breakthrough-infections occurring in patients receiving adequate mould-active prophylaxis is reason for concern. Investing time and resources to reduce the incidence or to decrease mortality is therefore warranted.

Figure 1: Ongoing developments and challenges in the field of invasive aspergillosis



Risk factors for invasive aspergillosis

Being aware of clinical risk factors for the development of IA makes it possible to employ prevention strategies more efficiently. Knowledge of the fact that patients with prolonged

neutropenia are at high risk for IA has led to the introduction of chemoprophylaxis in certain groups of patients with haematological malignancies (4-6). Additionally, knowledge of risk factors can support the clinician in the decision to initiate antifungal therapy or to perform additional diagnostics, e.g. analysis of bronchoalveolar lavage (BAL) fluid.

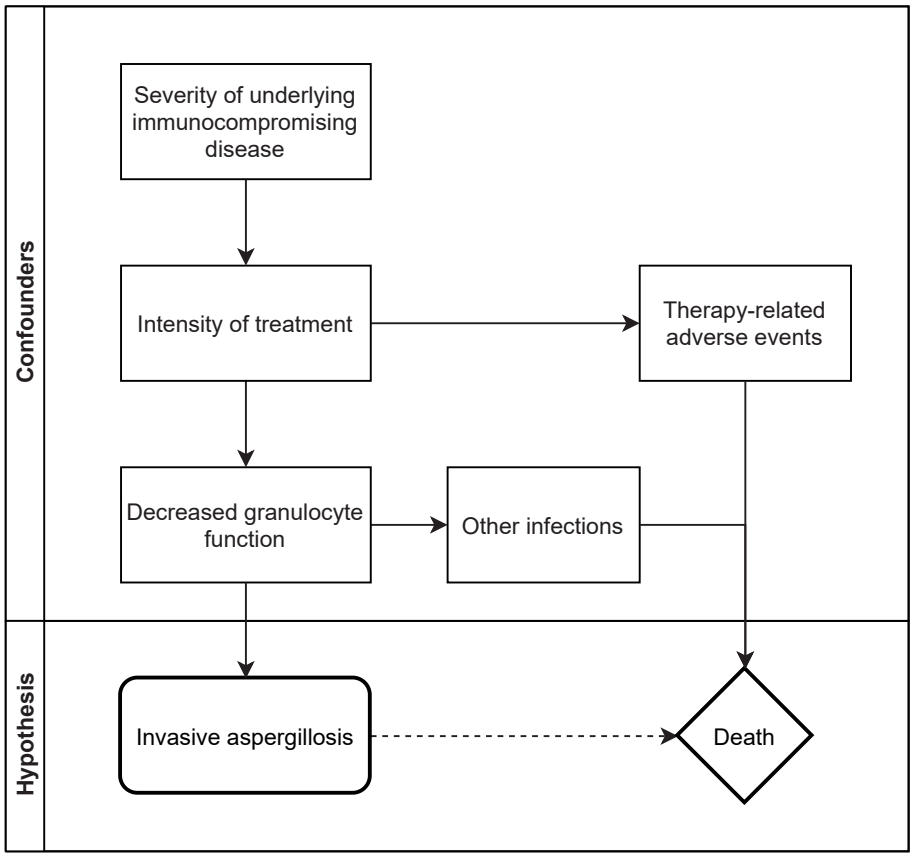
It is currently generally advised that patients who receive chemotherapy to induce remission of haematological disease (remission-induction therapy) in order to prepare them for allogeneic haematopoietic stem cell transplantation (HSCT) should receive antifungal chemoprophylaxis with activity against *Aspergillus* (1, 2, 6-8). In **chapter 3**, we have described the results of an analysis of risk factors for IA in patients receiving remission-induction therapy. Relapsed or secondary haematological malignancy and prolonged neutropenia were the most important risk factors for IA. It was not possible to identify and select patients within the study population with a very low risk of IA for whom chemoprophylaxis could be safely withheld. The results however did contribute to a more accurate identification of patients at high risk of IA and confirmed the need of a uniform application of antifungal chemoprophylaxis in patients receiving remission-induction therapy. Furthermore, it brings a further nuance to the results of chapter 2, in which only general populations at risk were described. It is apparent that the risk of IA within the group of patients with haematological malignancy can differ considerably between individuals.

Time relation and causal relation between invasive aspergillosis and mortality.

To reliably assess the impact of IA on health, it is necessary to gain insight in the way the infectious disease and mortality are linked. Mortality rates are often reported in the form of 1-year mortality, 100-day mortality or 30-day mortality rates. In the field of infectious diseases, the choice of the rate is dependent on the expected duration of disease. For example, the survival of HIV-infected individuals can generally best be expressed over a long period of time due to the prolonged asymptomatic phase and due to the present inability to cure patients from the infection. In the case of bacterial pneumonia, mortality rates could best be expressed in a short period of time due to the typically short and severe course of disease.

It is yet unclear whether IA has a mainly direct impact on mortality or can results in a prolonged negative impact on survival. Some studies have described that the excess mortality risk remains even long after the acute episode of IA. There are several possible explanations such as that HSCT is postponed during active IA, or that damage to the pulmonary tissue or other affected organs can have a permanent effect. Alternatively, it is possible that IA and mortality both have a common cause but are not causally linked in the long term. Common causes, i.e. a factors that both predispose a study subject for the risk factor of interest and the outcome of interest, introduce confounding to the results. In figure 2, several simple common causes that can confound the causal relationship between IA and death are schematically shown in a directed acyclic graph (DAG) (9).

Figure 2: Schematic illustration of the relationship between invasive aspergillosis and death with different confounders in a directed acyclic graph (DAG).



Legend: Arrows represent causal relations. The dotted arrow represents the relationship of interest.

To adjust for the confounding that has been introduced and identified, it is important to account for the effect of the common cause within the design of the statistical analysis. To reliably do this, it is necessary to quantify all common causes that can have an effect. This is a complex task in this patient population, due to the ever-changing state of the immune system of the patient at risk. Receiving chemotherapy, HSCT, suffering from treatment-related neutropenia are factors that change on a day-by-day basis and predispose for both IA and death.

In **chapter 4**, a time-dependent analysis is presented, showing the temporal relationship between IA, mortality, attaining HSCT, and daily changing risk factors. The results show a strong relationship between IA and death within the first 60 days after diagnosis, independent from the measured confounders. Long-term mortality does not seem to differ between patients who had suffered from IA.

In **chapter 7**, we have reviewed patients who died within 100 days after being diagnosed with IA. An independent committee judged the fatal cases to be attributable or not-attributable to IA. Is was found that in only a minority of cases, death could be (partially) attributed to IA. This suggests that patients with a high risk of dying from haematological malignancies or other complications of chemotherapy or HSCT also have a higher risk of developing IA. Subsequently, IA did not necessarily contribute to the cause of death.

Resistance against triazoles and antifungal stewardship

The use of voriconazole as initial therapy has become standard of care around the world due to high efficacy and relatively high tolerability. Emerging of resistance of *Aspergillus* fungi against triazole formulations is currently threatening the sustainability of the application of this drug as standard of care. Consequently, the use of liposomal amphotericin B (LAmB) as empiric therapy has increased (10-17). Antifungal stewardship is important to rationally use the available drugs we have and limit toxicity and costs while optimizing outcomes. Diagnostic modalities to detect resistance often fail, resulting in a majority of cases of IA in which triazole-resistance cannot be ruled out. Fear of starting ineffective treatment for an infection with high mortality could persuade the clinician to start treatment with LAmB instead of with the treatment of first choice, voriconazole. If the risk of resistance is high enough, this is a rational decision. It is however a difficult task to quantify the break-even point on which the benefits outweigh the risks of this treatment strategy, as this point is dependent on a multitude of different variable factors.

Recently introduced PCR techniques could help with the identification of triazole-resistant IA and improve the application of targeted therapy (3, 6, 18, 19). **Chapter 5** shows the impact of different therapeutic strategies in a simulated population, aided by improved resistance detection by PCR. The simulated outcomes for treatment with LAmB in case of unknown resistance is evaluated. The optimal cut-off point is highly dependent on the difference in efficacy between voriconazole and LAmB. The study that introduced voriconazole as an alternative treatment suggests that the efficacy of voriconazole is high (up to a 20% survival benefit) when compared to conventional amphotericin B (cAmB) (20, 21). Unfortunately, no direct comparisons between voriconazole and the liposomal formulation of amphotericin B (LAmB) have been made. Later studies applying treatment with LAmB report survival rates similar to those of voriconazole, or only slightly lower, although these data are neither randomised nor provide a direct comparison with voriconazole (22-27). The simulated data show the optimal cut-off points of background resistance rates from which point LAmB would provide the best outcome in case of unknown resistance. Depending on the difference in survival rates between LAmB and voriconazole, this cut-off point can be either as low as 6% or as high as 20%.

There is an increased need for prolonged treatment with LAmB. The high rates of nephrotoxicity and unavailability of an oral formulation make the drug an unattractive option for

treatment outside of the hospital. Nonetheless, there is currently no alternative option for patients that are intolerant of triazoles or are suffering from triazole-resistant IA.

In **chapter 6**, we showed the characteristics and outcomes of 18 patients treated for an invasive fungal infection with LAmB in a two- or three-times weekly dosing regimen in an outpatient setting. Our findings support the hypothesis that LAmB can be safely applied in this setting, under the condition that kidney function is monitored regularly during the course of treatment due to the high prevalence of renal toxicity.

Chapter 7 introduced a clinical decision rule for the management of IA. The decision rule aims to identify patient groups that benefit from treatment with LAmB instead of voriconazole in case of unknown triazole susceptibility. We have shown that the decision tool successfully limits the use of LAmB and of combination therapy with both voriconazole and LAmB while still maintaining a case fatality rate that is in line with the rates mentioned in literature. Furthermore, later switch from voriconazole to LAmB because of later demonstrated triazole resistance or insufficient treatment response was not associated with a higher mortality when compared to patients that initially started therapy with LAmB. It was shown that mortality directly attributable to IA was low when compared to overall mortality, accounting for less than 50% of the 100-day mortality.

Use of observational data to improve the management of invasive aspergillosis

Evidence-based guidelines are periodically published to help clinicians all over the world in the management of IA. However, many recommendations are not supported by the gold standard in evidence-based medicine, the randomised controlled trial (RCT). Retrospective research, non-comparative prospective studies, case-control studies, case series and expert opinions are the main basis of recommendations in the most important international guidelines (1-3). In theory, a well-designed, high-powered RCT could compare many different management strategies and identify the optimal one. However, concerns of time and costs make this option not very realistic. The time required to design an RCT and recruit study participant of this relatively rare disease would be very long and may even exceed the duration in which the study results are applicable in practice due to changing epidemiology, diagnostics and treatment options. Additionally, the external validity of RCTs is often hampered by strict in- and exclusion criteria, and the results may not be readily applicable in the multimorbid patient. The highly dynamic interaction between the pathogen, the host, diagnostics and therapy, necessitates flexible alterations in the management for the individual patient.

In this thesis, I have mainly described research dependent on retrospective data. This approach has several strengths and limitations. First, the retrospective data used in this thesis provide a very recent, low-cost view of the current dynamics in the field of IA. Second, the retrospective data show a more complete part of the patient population, whereas RCTs are often accompanied by the shortcomings that certain patient groups are excluded, or not willing to participate. The main disadvantages that are introduced by the observational nature of the

studies, are some sources of confounding that can be hard to control for. For example, to compare the outcomes of two different methods of treatment can be biased due to confounding by indication. One of the strong characteristics of randomized controlled trials is that they account for this source of bias by randomizing treatment allocation.

Chapter 5 showed us an example of a modelling study. Modelling studies can provide insight using available data to evaluate clinical applications in the current clinical landscape, and even make predictions about how our conclusions might change when important epidemiological factors are altered. These kind of studies are of course limited by the quality of the data they are based on; if weak evidence is used to construct the model, the outcomes may not be as robust. However, although no new, original data is added to our knowledge base, it can make the application of current knowledge in changing circumstances insightful.

Conclusions and future challenges

From all the previous chapters, we have learned that despite developments in the prevention of IA, the incidence rates are up to 10% in high risk groups. Within high risk groups, individual patients with an even higher risks can be identified, such as those suffering from relapsed AML. Mortality of IA is high within all populations and is mainly found in the first 30 days after diagnosis. Because of the serious nature of the underlying diseases that predispose for IA, it can be challenging to quantify the contribution of IA to mortality; crude mortality rates cannot distinguish death due to IA-unrelated causes from IA-related death, almost certainly leading to inflation of mortality rates. It can be helpful to assess contributability of IA to death to provide a better understanding of the impact of IA in this vulnerable patient population.

Additionally, we have explored the new problem that is posed by the increase of triazole-resistant *Aspergillus* fungi, resulting in challenges in the application of antifungal therapies. Treatment strategies involving the use of LAmB are becoming more prevalent in areas with high resistance rates, but should be applied with care due to concerns of renal toxicity and decreased efficacy against triazole-susceptible isolates when compared to voriconazole. Rational application of PCR could help us to initiate the right therapy sooner, by possibly providing information about the susceptibility of the *Aspergillus* fungus.

Application of the results from the works published in this thesis can be of use in antifungal stewardship policies and optimizing personalised treatment, diagnostic and prevention strategies. The field of invasive fungal infections has thus far always been hindered by small patient populations and a low number of randomised studies. To find a definitive answer on the impact of triazole resistance rate and an unbiased analysis of mortality attributable to IA, large multicentre randomised studies are needed. Alternatively, further research could use large volumes of observational data, possibly extractable from already existing registries. Although these data are not randomised, valuable insights about the current practice of antifungal management could be obtained from such "big data" studies.

We must try to limit the environmental pressure on *Aspergillus* fungi to develop mutations that provide triazole resistance. This pressure has been linked to the extensive use of fungicides, mainly in agriculture (28). Due to the high mobility of fungal spores, resistance mutations can easily travel the whole world and the negative effects this has have already been reported (29-35). In order to limit the impact of environmental fungicide use on resistance selection, it is necessary to apply a "One Health" approach featuring extensive collaboration between clinicians, epidemiologists, biologists, agricultural science and governments.

At present, there are multiple problems regarding the appropriate use of antifungal drugs. Chemoprophylactic strategies provide incomplete protection against IA, and both prophylactic and therapeutic use of triazoles is hampered by unreliable serum drug levels, toxicity, drug-drug interactions and triazole-resistance. LAmB is mainly limited by the intravenous application, renal toxicity and high costs. Additionally, patients with triazole resistant IA who are intolerant of LAmB have no good alternative treatment option. A new drug could improve upon one or more of these shortcomings. Further research should explore further application of echinocandins or the development of an entirely new class of drugs that does not suffer from the renal toxicity of LAmB or the rising triazole resistance rates. The recent introduction of olorofim shows promise in fulfilling the role of an oral alternative in this case (36-39). Clinical experience with this drug is still limited, but in potential it might be able to meet one of the most urgent needs in the treatment of IA.

In conclusion, new chemoprophylactic or therapeutic strategies and the rational application of diagnostic methods are needed to overcome the challenges we are facing in the management of IA.

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Nederlandse samenvatting

INTRODUCTIE

In de afgelopen tientallen jaren zijn er belangrijke ontwikkelingen geweest in de behandeling van mensen met levensbedreigende vormen van kanker. In de jaren 50 van de vorige eeuw is de hematopoïetische stamceltransplantatie (HSCT) geïntroduceerd en met succes toegepast om verschillende vormen van acute leukemie te genezen. Bij deze behandeling wordt met behulp van een intensieve chemokuur het eigen afweersysteem in het beenmerg gedood en kan een nieuwe lijn van witte bloedcellen zich ontwikkelen uit stamcellen die afkomstig zijn van een donor. De levensverwachting en kwaliteit van leven bij deze patiënten heeft mede door de goede resultaten van de intensieve behandelingen een flinke sprong voorwaarts gemaakt.

Het traject van intensieve chemotherapie en HSCT is langdurig, en gaat gepaard met een maandenlange verminderde werking van het afweersysteem. Gedurende deze periode zijn mensen gevoelig voor ziekteverwekkers waar mensen met een gezond immuunsysteem niet vatbaar voor zijn. Infecties veroorzaakt door deze ziekteverwekkers worden opportunistische infecties genoemd. Een belangrijke opportunistische infectie die met name veel voorkomt bij patiënten die behandeld worden voor kanker van de witte bloedcellen, is invasieve aspergillose (IA). Dit is een ziekte die zich meestal uit als een longontsteking en wordt veroorzaakt door een schimmel van het geslacht *Aspergillus*.

Aspergillus-schimmels produceren sporen die zich eenvoudig door de lucht kunnen verspreiden en kunnen uitgroeien tot schimmeldraden indien zij een omgeving bereiken met de juiste beschikbaarheid van voedingsstoffen en de juiste temperatuur. Deze schimmeldraden produceren op hun beurt weer nieuwe sporen. De sporen van *Aspergillus*-schimmels komen van nature overal voor, in zowel de binnen- als buitenlucht. De schimmel heeft ook een belangrijke taak bij het opruimen van organisch materiaal. Het geslacht *Aspergillus* bestaat uit honderden verschillende soorten waarvan elke soort bepaalde eigenschappen bezit waardoor zij een voorkeur voor een bepaald soort voeding, regio of klimaat hebben. In Europa en de Verenigde Staten is *Aspergillus fumigatus* de meest gebruikelijke verwekker van ziekte bij mensen. Andere soorten die ziekte bij de mens kunnen veroorzaken zijn onder andere *A. flavus, A. niger en A. terreus*. Tussen onderlinge soorten kan bijvoorbeeld de gevoeligheid voor medicatie of het groeipatroon verschillen. Ziekten die worden veroorzaakt door soorten van deze schimmel worden veelal aangeduid met de term 'aspergillose'.

Het ziektebeeld invasieve aspergillose

Schimmels van het geslacht *Aspergillus* kunnen verschillende soorten ziekten veroorzaken bij de mens. In dit proefschrift beperk ik mij tot de meest ernstige vorm: invasieve aspergillose (IA). Het spectrum van de verschillende ziekten van aspergillose wordt bepaald door de interactie tussen de ziekteverwekker en de gastheer. Allergische bronchopulmonale aspergillose (ABPA) wordt gekenmerkt door een allergische of overgevoeligheidsreactie op de schimmel. Een aspergilloom of een chronische cavitaire pulmonaire aspergillose is een chronische kolonisatie door

schimmeldraden, meestal optredend in de long bij mensen met een eerdere longaandoening. Bij deze vormen van aspergillose treedt er geen invasieve groei van schimmeldraden op, dat wil zeggen dat de schimmel de gezonde weefsels van de mens niet kan doordringen. Dit gebeurt alleen bij IA, die uitsluitend op kan treden bij patiënten met een verzwakte immuniteit. Aangezien de ademweg de meest eenvoudige route is voor de schimmel om het lichaam binnen te dringen, uit de ziekte zich vaak als een ontsteking van de long of van de neusbijholten. Vanuit een van deze ontstekingshaarden is het mogelijk dat de schimmel via de bloedbaan op een plek elders in het lichaam kan uitgroeien en ter plaatse een tweede focus van invasieve infectie vormt. Als de infectie niet bestreden wordt door de afweercellen van de gastheer of door medicatie, zal deze zich uitbreiden en uiteindelijk tot de dood leiden door een (long) bloeding of ernstige ontstekingsreactie.

Het herkennen en behandelen van invasieve aspergillose

De neutrofiele granulocyt, een van de subtypen van de witte bloedcel, vormt de belangrijkste verdediging tegen invasieve groei van *Aspergillus*-schimmels. Met name patiënten die worden behandeld met intensieve chemotherapie hebben een verminderd aantal of functie van de neutrofiele granulocyt. Als deze patiënten klinische verschijnselen krijgen die kunnen passen bij IA, waarvan koorts de belangrijkste is, moet de arts diagnostiek inzetten om de diagnose IA meer of minder waarschijnlijk te maken. Er kan dan worden gekozen om een röntgenscan (CT-scan) te maken van de longen, of om een diagnostische longspoeling (een broncho-alveolaire lavage ofwel BAL) of longbiopt te laten verrichten door de longarts.

Het materiaal dat door de BAL wordt verkregen kan worden onderzocht door de arts-microbioloog op tekenen van de aanwezigheid van de *Aspergillus*-schimmel. Dit kan op drie manieren:

1. Het aantonen van galactomannaan, een bestanddeel van de celwand van de schimmel.
2. Het kweken van de schimmel, dat wil zeggen het aanbrengen van de BAL-vloeistof op een kweekmedium, en af te wachten of er een schimmel gaat groeien die verder getypeerd kan worden. Ook kan dan getest worden of de gekweekte schimmel gevoelig is voor de antibiotica die werkzaam zijn tegen schimmels (deze middelen worden ook wel antifungale of antimycotische middelen genoemd).
3. Het aantonen van de aanwezigheid van DNA van de *Aspergillus*-schimmel met behulp van een methode die polymerasekettingreactie (PCR) heet. Ook kan in het DNA van de schimmel gekeken worden of er mutaties aanwezig zijn die ervoor zorgen dat de schimmel ongevoelig kan zijn voor antifungale middelen.

In het longbiopt kan met zekerheid worden vastgesteld of er sprake is van IA, aangezien hier de relatie tussen de schimmeldraden en het menselijk weefsel kan worden geobserveerd. Het longbiopt wordt voor deze indicatie slechts zelden toegepast aangezien het risico op een ernstige complicatie zoals een longbloeding vaak te hoog wordt geacht bij de doorgaans ernstig

zieke patiënt die verdacht wordt van IA. Alle andere vormen van diagnostiek gaan echter gepaard met een bepaalde onzekerheid over de aanwezigheid van IA.

Als de behandelaar de diagnose IA voldoende aannemelijk vindt, kan hij de ziekte behandelen met medicijnen. Ook kan er heel soms gekozen worden om aangedaan weefsel met een operatie te verwijderen. Het belangrijkste medicijn in de behandeling van IA is het antifungale middel voriconazol. Dit middel valt samen met een aantal andere middelen in de groep van de triazolen, die een belangrijk bestanddeel van de celwand van de schimmel kunnen afbreken. In geval van resistentie voor triazolen kan er gekozen worden voor liposomaal amfotericine B (LAmB), een middel dat waarschijnlijk minder effectief is dan voriconazol en meer ernstige bijwerkingen heeft. Bij patiënten waarvan het risico op IA al bij voorbaat als sterk verhoogd wordt ingeschat, kan er voor gekozen worden om preventief te behandelen met een antifungaal middel zoals voriconazol. Deze zogenaamde profylactische toepassing van antifungale middelen wordt steeds vaker ingezet en is de standaard geworden voor mensen die een HSCT ondergaan.

Resistentieontwikkeling

Behandeling van IA wordt bemoeilijkt door een progressieve toename van gevallen waarin de schimmel resistent is tegen triazolen. Voriconazol behoort tot deze groep middelen en is doorgaans in de nationale en internationale richtlijnen de eerste keus als behandeling van IA. Vanwege deze resistentieontwikkeling moet er steeds vaker gekozen worden voor behandeling met LAmB. De ontwikkeling van resistentie is in de literatuur gelinkt aan de uitgebreide toepassing van triazolen als bestrijdingsmiddel in de agrarische sector.

DIT PROEFSCHRIFT

In **hoofdstuk 2** beschrijven we een uitgebreid systematisch literatuuronderzoek om meer te weten te komen over de frequentie van het optreden van IA en de hierop volgende sterfte. Deze informatie is belangrijk om de impact van IA op de volksgezondheid in te schatten en zo de urgentie voor nieuwe behandelmethoden aan te tonen. Ook is deze informatie van belang om de effecten van strategieën om de ziekte te voorkomen of te genezen te evalueren.

Het literatuuronderzoek omspant alle publicaties gedurende een periode van 9 jaar die een studiepopulatie omschrijven met een verhoogd risico op het ontwikkelen van IA. Binnen deze studies is gekeken naar de onderliggende aandoeningen van deze patiënten en of zij wel of niet profylactisch antifungale medicatie hebben gekregen. Een belangrijke bevinding is dat IA optreedt bij tot wel 11% van alle patiënten die behandeld worden voor een ernstige hematologische maligniteit. Het toepassen van antifungale profylaxe zorgt ervoor dat de kans op het optreden van IA wordt verkleind, maar in de hoog-risico populaties blijft een risico van tenminste 6% aanwezig. De kans op sterfte op korte termijn na het ontwikkelen van IA wordt op 29% geschat op grond van de aanwezige gegevens. Samenvattend blijft er ondanks de

toepassing van profylaxe en behandeling met antifungale medicijnen een groot risico aanwezig op het ontwikkelen van IA en is er een grote kans op sterfte bij patiënten met IA.

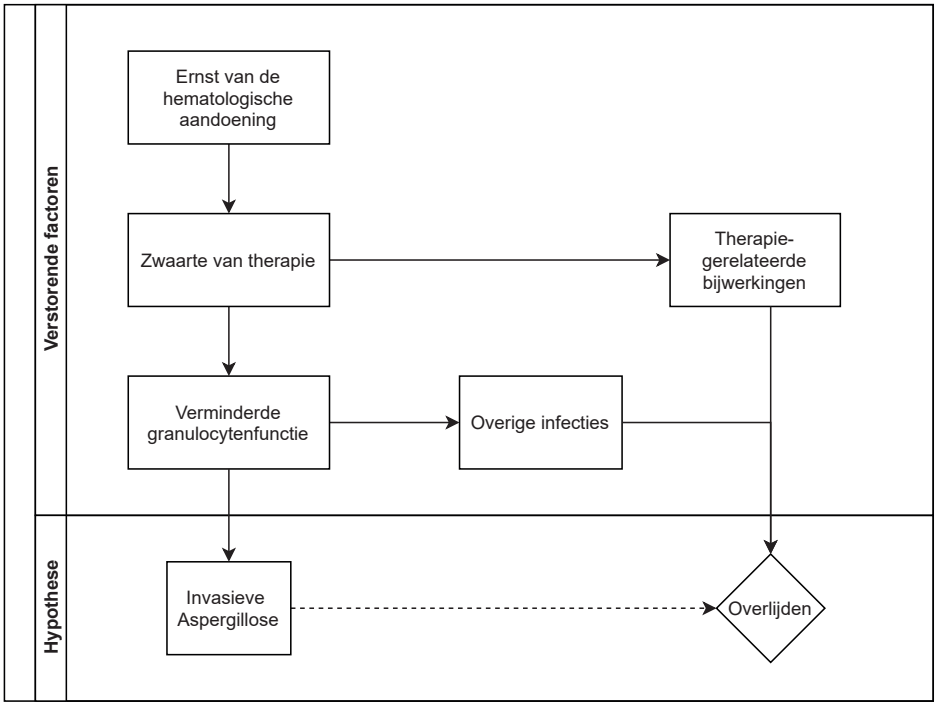
Hoofdstuk 3 gaat verder in op de vraag hoe goed we vooraf het risico op het krijgen van IA kunnen inschatten. Het is bekend dat het risico op IA verhoogd wordt door bepaalde patiëntgebonden factoren, zoals een hogere leeftijd en het hebben van een laag aantal neutrofiele granulocyten. Door alle risicofactoren in kaart te brengen zou het mogelijk kunnen zijn om een voor de individuele patiënt toepasbare risico-inschatting te maken.

Uit de analyse van 167 patiënten met een hematologische maligniteit kwam naar voren dat onder andere patiënten die eerder voor een hematologische of andere vorm van kanker zijn behandeld een duidelijk verhoogd risico op IA hadden. Op grond van de analyse was het niet mogelijk om een selectie van patiënten te maken die een zeer laag risico op IA hebben, en daarom geen profylaxe zouden hoeven gebruiken. De resultaten van de studie kunnen de behandelaar helpen om de aannemelijkheid van de diagnose IA in te schatten en om eventuele geïndividualiseerde patiëntstrategieën voor de preventie van IA te ontwikkelen.

Hoofdstuk 4 beschrijft de relatie tussen IA en sterfte in meer detail. Deze relatie is complex omdat patiënten die IA ontwikkelen al ernstig ziek zijn aangezien ze vaak een moeilijk behandelbare vorm van kanker hebben. De sterftekans van deze patiënten zal hoog zijn, ook als zij geen IA ontwikkelen. Om een analyse uit te kunnen voeren waar de relatie tussen IA en sterfte wordt onderzocht, is het van belang om factoren te identificeren die een verstorende werking kunnen hebben. Mensen die ernstiger ziek zijn, bijvoorbeeld omdat de hematologische maligniteit onvoldoende gereageerd heeft op de chemokuur, hebben een grotere kans om te overlijden aan de hematologische aandoening zelf. Deze patiënten hebben echter, zoals beschreven in hoofdstuk 3, tevens een verhoogd risico op het ontwikkelen van IA. De respons van de hematologische maligniteit op de kuur wordt om deze reden gezien als een verstorende variabele, vaker aangeduid met de uit de Engelstalige literatuur afgeleide term *confounder*. In de studie zijn zoveel mogelijk denkbare confounders geïdentificeerd en hun invloed vervolgens geëlimineerd door het ontwerp van het analytisch model. Belangrijke confounders zijn de ontvangen therapie, de ernst van de onderliggende (hematologische) aandoening en de functie van de neutrofiele granulocyten van de patiënt. In figuur 1 wordt op een vereenvoudigde, schematische wijze een overzicht weergeven van de manier waarop enkele confounders van invloed kunnen zijn bij het onderzoeken van de oorzakelijke relatie tussen IA en overlijden. Uit de figuur kan bijvoorbeeld worden opgemaakt dat het geven van een zwaardere therapie zowel leidt tot een hoger risico op IA als op een hoger risico op overlijden, onafhankelijk van het optreden van IA. Door deze verstorende factor kan het oorzakelijke verband tussen IA en overlijden worden overschat.

Ook na het corrigeren voor de confounders blijkt uit de uitgevoerde berekening dat er een groot risico op sterfte is ten gevolge van IA, en dat het risico het grootst is binnen de eerste 60 dagen na diagnose. Na het overleven van de eerste 60 dagen is er geen merkbaar nadeel in de overleving van patiënten die IA hebben doorgemaakt.

Figuur 1. Een vereenvoudigde, schematische weergave van een aantal belangrijke verstorende factoren (confounders) die meespelen bij het onderzoeken van de oorzakelijke relatie tussen invasieve aspergillose en overlijden.



Legenda: Pijlen geven een oorzakelijk verband aan, de gestippelde pijl geeft het vermeende oorzakelijk verband aan dat in de studie wordt onderzocht.

Hoofstuk 5 beschrijft een theoretisch model van de toepassing van resistentietypering met behulp van PCR. Deze methode van resistentiebepaling heeft een hogere slagingskans en kost minder tijd dan het bepalen van de gevoeligheid voor antifungale middelen door een kweek. Algemene toepassing van deze techniek kan ervoor zorgen dat patiënten met IA die wordt veroorzaakt door een *Aspergillus*-schimmel met resistentie tegen triazolen eerder de juiste therapie ontvangen en niet het risico lopen om met het in dat geval ineffectieve voriconazol te worden behandeld. Het beschreven model laat zien dat er een belangrijke gezondheidswinst is bij een effectieve testmethode als het percentage triazolresistentie hoog is. Ook laat het model zien welke factoren er betrokken zijn bij de afweging voor het juiste middel in het geval dat de testen ons in de steek laten en er geen resistentietypering beschikbaar is. De voorafkans op triazol-resistentie is daarin van grote invloed in de beslissing om te starten met voriconazol of met LAmB. Indien het triazolresistentie-percentage 6% of hoger is kan het verstandig zijn om in afwachting van de resistentiebepalingen te starten met het tweede keus-middel LAmB. Het precieze afkappunt vanaf welke resistentiekans dit verstandig is, is afhankelijk van het daadwerkelijke overlevingsvoordeel van voriconazol boven LAmB bij een triazol-gevoelige IA.

Uit meerdere studies zijn gegevens bekend van dit overlevingsvoordeel, maar er is nog steeds een discussie over de grootte van het voordeel.

Het uitvoeren van een modelstudie zoals deze heeft meerwaarde, omdat het in de praktijk uitvoeren van een dergelijke grote prospectieve studie zeer kostbaar zou zijn en vele jaren in beslag zou nemen. De situatie met betrekking tot resistentiepercentages en nieuwe antifungale middelen zal dan alweer veranderd zijn.

In **hoofdstuk 6** beschrijven we 18 patiënten uit meerdere centra in Nederland en België die poliklinisch behandeld worden met LAmB. Vanwege een toenemende toepassing van dit middel is er behoefte aan nieuwe manieren om het te gebruiken. Omdat het alleen per infuus kan worden gegeven, is het gebruik ervan normaal gesproken beperkt tot patiënten die in het ziekenhuis zijn opgenomen. Voor zover bekend was een poliklinische behandeling nergens ter wereld een onderzochte en aanvaarde wijze van behandelen, omdat behandelaren de potentiële risico's te groot achtten. Ook binnen het consortium van de Dutch-Belgium Mycosis Study Group waarbinnen deze studie is uitgevoerd was er slechts een beperkt aantal centra die de poliklinische behandeling wel eens toepasten. De bevindingen uit het onderzoek laten zien dat het veilig en effectief is om het middel 2 tot 3 keer per week in een poliklinische setting toe te passen, in plaats van dagelijks in de kliniek. Wel is het belangrijk om de nierfunctie strikt te blijven monitoren vanwege de grote kans op nierfunctiestoornissen veroorzaakt door LAmB.

Ten slotte beschrijven we in **hoofdstuk 7** de introductie van een nieuwe behandelregel voor patiënten met IA. Recent is een nationaal advies verschenen om in centra waar triazol-resistente schimmels veel voorkomen, zoals in de regio van het Leids Universitair Medisch Centrum, behandeling te starten met zowel voriconazol als LAmB in afwachting van de resistentie-analyse. De rationale van dit advies is gebaseerd op de overweging dat voriconazol een superieure overleving geeft ten opzichte van LAmB, maar in geval van resistentie niet effectief is.

Dubbeltherapie met deze middelen heeft echter belangrijke nadelen. Beide middelen hebben belangrijke bijwerkingen, waarvan LAmB over het algemeen de meest ernstige kan geven. Daarnaast heeft voriconazol invloed op de effectiviteit van andere medicijnen en kunnen de medicijnconcentraties in het bloed onvoorspelbaar zijn. Een andere beperking is dat LAmB uitsluitend via een infuus kan worden toegediend. Ook is het in de praktijk vaak lastig om de stap te zetten om één van de middelen te staken aangezien resistentiebepaling slechts in een minderheid van de gevallen lukt.

Naar aanleiding van de twijfels over de meerwaarde om deze dubbeltherapie te starten is er in het Leids Universitair Medisch Centrum gekozen om een beslisregel te ontwikkelen die de behandelaar helpt om veilig een geschikt middel als enkelvoudige therapie te kiezen. Om het risico te ondervangen dat patiënten met een triazol-resistente IA worden onderbehandeld, is ervoor gekozen om patiënten met een hoger risico op een ernstig beloop van IA of met een potentieel verhoogde kans op de aanwezigheid van een triazol-resistente IA initieel te behandelen met LAmB. Bij afwezigheid van deze factoren wordt gekozen voor behandeling met voriconazol. Uit de resultaten van de studie kan worden afgeleid dat het effectief is om

de geselecteerde patiënten initieel met voriconazol te behandelen, ondanks het hoge risico op resistentie in Nederland. Een sterk methodologisch punt van deze studie is dat er aandacht is besteed aan het definiëren van de oorzakelijke relatie tussen IA en sterfte. Het vaststellen van een oorzakelijk verband wordt bemoeilijkt door de vele confounders (zie ook figuur 1). Voor deze studie heeft een groep van expert-behandelaars vastgesteld wanneer een overlijden aan IA is gerelateerd. Uit deze uitkomsten kunnen we aannemelijk maken dat een groot deel van de overlijdens na het optreden van IA niet gerelateerd blijkt aan IA.

CONCLUSIES

De problemen bij de behandeling en preventie van IA zijn met behulp van de resultaten uit dit proefschrift beter in kaart gebracht. Uit het literatuuronderzoek is gebleken dat antifungale profylaxe voor patiënten met een hoog risico op IA lang niet alle gevallen van IA kan voorkomen. Dit blijft een belangrijke overweging bij het nemen van beslissingen over diagnostiek en empirische behandeling. Onderzoek naar de relatie tussen IA en overlijden heeft verschillende inzichten opgeleverd, namelijk dat (1) de "ruwe" schatting van overlijden volgend op IA 23% van alle gevallen betreft, en (2) de relatie tussen IA en sterfte zwakker wordt na verloop van tijd en na 60 dagen niet meer duidelijk aantoonbaar is en (3) dat veel factoren bij de doorgaans ernstig zieke patiënt met IA het onderzoek naar deze relatie verstoren en zorgen voor een overschatting van de oorzakelijke sterfte.

Verder is resistentie van *Aspergillus*-schimmels tegen triazolen, een van de meest veilige en effectieve middelen tegen IA, reden tot zorg. Slimme inzet van diagnostische middelen kan bijdragen aan vroege herkenning van resistentie en ervoor zorgen dat de juiste therapie snel wordt toegepast. Door de toepassing van adequate diagnostiek en een risico-inschatting op het moment van diagnose kan er veilig gekozen worden tussen LAmB en voriconazol in plaats van een combinatie van deze middelen. Ook is het veilig om LAmB in een schema van enkele keren per week toe te dienen onder strikte monitoring van de nierfunctie.

Verder onderzoek over slimme toepassing van het beperkte arsenaal aan beschikbare middelen kan hopelijk bijdragen aan betere uitkomsten in het voorkomen en behandelen van IA. Momenteel wordt er veelbelovend onderzoek verricht naar de werkzaamheid van olorofim, een middel met een geheel nieuw mechanisme van antifungale activiteit. Door de tekortkomingen van de huidige therapieën is er een hoge nood voor de ontwikkeling van een nieuw middel met minder ernstige bijwerkingen, dat niet alleen per infuus beschikbaar is en waarvoor nog geen resistentie bestaat.

Curriculum Vitae

Robert Jan van de Peppel werd geboren op 28 mei 1992 in Assen. Na het behalen van het vwo-diploma aan het Stedelijk Gymnasium in Schiedam begon hij in 2009 aan de studie Geneeskunde in Leiden. Gedurende de bacheloropleiding werd in de collegezaal zijn interesse gewekt voor de interne geneeskunde en in het bijzonder de klinische infectieziekten. Na een ontmoeting met dr. Mark G.J. de Boer, zijn latere copromotor, begon hij een wetenschappelijke stage om factoren te kunnen identificeren die het risico op invasieve aspergillose verhogen. Gedurende de opleiding heeft hij extra nadruk gelegd op verdieping in de interne geneeskunde, infectieziekten, medisch onderwijs en klinische epidemiologie. Ter afronding van de opleiding geneeskunde heeft hij zijn semi-artsstage gevolgd bij de afdeling interne geneeskunde in het HagaZiekenhuis in den Haag (opleider dr. ir. Joep Lagro) waarna in 2016 het artsexamen is afgelegd. Vervolgens is het onderzoek voortgezet in de vorm van een promotietraject met prof. dr. Leo G. Visser als promotor en dr. Mark G.J. de Boer als copromotor. Deels is dit traject gevolgd op de afdeling klinische epidemiologie onder begeleiding van prof. dr. Frits R. Roosendaal en prof. dr. Olaf M. Dekkers.

Na het onderzoekstraject persisteerde zijn wens om zich verder te bekwamen in de interne geneeskunde. Om de klinische kant van het vak te ontwikkelen, begon hij in 2018 als arts-assistent interne geneeskunde in het Alrijne Ziekenhuis in Leiderdorp (opleider drs. Sander Anten). In 2019 volgde acceptatie voor de opleiding tot internist in het LUMC (opleider prof. dr. Johan (Hans) W. de Fijter en prof. dr. Leo G. Visser). In 2020 startte hij met de opleiding in de perifere opleidingskliniek het Groene Hart Ziekenhuis in Gouda (opleider dr. Ted Koster). In hetzelfde jaar is Robert getrouwd met Annemieke Stolk en samen wonen zij in Leiden.

Nawoord

Veel verschillende mensen zijn mij tot ondersteuning of inspiratie geweest bij de totstandkoming van dit proefschrift.

Tijdens mijn jaren in de collegebanken, bij de studentenvereniging, als coassistent, als onderzoeker en momenteel als arts in opleiding tot internist zijn er ontelbaar veel collega's, docenten en goede vrienden geweest die mij gesteund hebben, in welke vorm dan ook. Het zou niet mogelijk zijn om al deze personen en hun individuele bijdragen hier te vermelden. Wel wil ik mijn copromotor Mark in het bijzonder noemen. Wat begon met een mailtje dat ik je stuurde na een van je inspirerende colleges, is uitgegroeid tot een prachtig project. Ik heb ontzettend veel van je geleerd, zowel op de afdeling Infectieziekten als tijdens de bijzondere congressen die ik met je bezocht heb. Daarnaast vond ik het altijd erg fijn om met je samen te werken en ben je een inspiratiebron voor hoe ik mij wil ontwikkelen als internist. Je hebt mijn diepe dank hiervoor.

Mijn lieve zussen, Judith, Wilke en Anne, hartelijk dank voor jullie steun en liefde. Wilke, jij bent mij voorgegaan bij de studie geneeskunde in Leiden en ik heb mede dankzij jouw goede voorbeeld datzelfde pad gevolgd. Net als bij onze voortgangstoetsen destijds, ben ik heel blij dat je nu ook bij mijn promotie naast mij wil zitten, ditmaal als mijn paranimf.

Maar bovenal ben ik mijn ouders veel dank verschuldigd. Hans en Anneke, jullie hebben een liefdevolle omgeving gecreëerd waarin ik heb mogen opgroeien. Jullie hebben mij altijd gestimuleerd om mijn ambities waar te maken en mij daarvoor de mogelijkheden gegeven. Ook mijn schoonouders Leo en Els wil ik graag bedanken, jullie zijn mij en Annemieke altijd tot steun geweest. Daarnaast hebben de geïnteresseerde gesprekken met Leo over de epidemiologie en farmacologie zeker bijgedragen aan mijn inspiratie tijdens het schrijven van dit werk.

Tot slot wil ik mijn lieve vrouw Annemieke bedanken. Wat ben ik blij dat ik met jou heb mogen trouwen, in een bewogen tijd aan de start van mijn opleiding tot internist en het afronden van mijn proefschrift. Gelukkig heb ik aan jou altijd een stabiele, liefdevolle partner en ben je al jaren mijn steun en toeverlaat. Ik kijk uit naar onze verdere mooie toekomst samen en binnenkort naar ons eigen gezin.

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