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

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Chapter 1

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

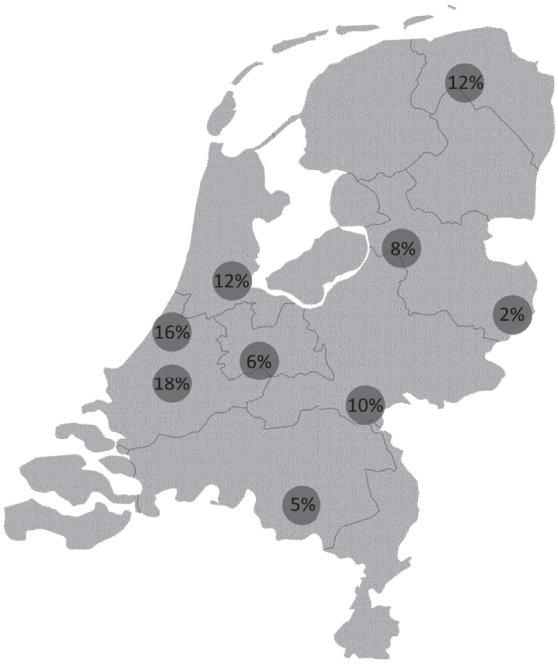
Class	Examples	Mechanism of action	Important safety considerations	Recommendation in guidelines	Resistance
Triazoles	Voriconazole Posaconazole Itraconazole Isavuconazole	Inhibitor of 14-alpha-demethylase, an enzyme necessary for the production of ergosterol, an important compound of the fungal cell wall.	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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