Chapter 1

General introduction and outline of the thesis

INTRODUCTION

Invasive aspergillosis (IA) is a severe opportunistic infection that is mainly caused by *Aspergillus fumigatus* [1,2]. It is the most common invasive fungal disease (IFD) in immunocompromised patients with an underlying hematological disease, including those with acute myeloid leukemia (AML) receiving remission induction or consolidation chemotherapy, and recipients of allogeneic hematopoietic stem cell transplantation (alloHSCT) [1,2]. Not only does it lead to an increase in morbidity and mortality among these patients [3,4], but also to an increase in medical costs [5]. Given these facts, it is essential to optimize management of IA (including early diagnosis, adequate antifungal therapy and prevention) in high-risk patients with an underlying hematological disease.

Currently, the first line therapy is voriconazole, an antifungal agent from the class azoles [6]. Before the introduction of mold-active azoles in the 1990s, mortality rates as high as 90% have been reported in patients with an underlying hematological disease [3,4]. When the recommended therapy with voriconazole is initiated promptly, a relatively low mortality between 26% to 39% at 12 weeks after diagnosis is observed [5,7,8]. Early diagnosis is therefore highly important to treat IA without any delay.

Another strategy in the management of IA is prevention through antifungal prophylaxis. Antifungal prophylaxis with azoles, echinocandins and polyenes have shown to be effective in preventing IA in high-risk patients with an underlying hematological disease [9-12]. At present, mold-active azoles posaconazole and voriconazole are recommended in patients with prolonged neutropenia or in allogeneic alloHSCT recipients with active graft-versus-host disease (GVHD) [6]. Besides reducing the incidence and IA-related mortality, antifungal prophylaxis may also be cost-effective. This thesis focuses on IA in high-risk patients with an underlying hematological disease and in particular on its epidemiology, prevention and diagnostics.

INVASIVE ASPERGILLOSIS: INCIDENCE, MORTALITY, RISK FACTORS AND PREVENTION

In the management of IA, it is important to monitor the local incidence and mortality in high-risk patients as these are subjected to continuous changes, such as alterations in antifungal prophylaxis and the emergence of azole-resistant *A. fumigatus* and intrinsically azole-resistant sibling species. Moreover, risk factors should be identified to recognize the subset of patients who are at highest risk and will benefit most from antifungal prophylaxis. **Chapter 2** describes the incidence, mortality and risk factors of IA in alloHSCT recipients who were transplanted in the Erasmus University Medical Center over the past decade.

As mentioned earlier, IA is not only associated with an increased morbidity and mortality, but also with a substantially increase of medical costs in patients with an underlying hematological disease [3-5,7,8]. Given these observations and the fact that hospital resources are not infinite, preventing IA is an attractive strategy to reduce IA-related mortality and costs. Although posaconazole and voriconazole are recommended as antifungal prophylaxis [6], other antifungals from other classes can be used as prophylaxis as well [10,12]. Inhalation of aerosolized liposomal amphotericin-B (L-AmB) has been shown to be effective and safe in preventing IA [10,13]. It has the advantages of (i) no systemic side effects in compare to intravenous L-AmB and other antifungal prophylaxis, and (ii) retaining azoles in case of therapy. In 2008, it was implemented as standard of care for patients with AML receiving intensive chemotherapy in the Erasmus University Medical Center. **Chapter 3** describes the efficacy and cost-effectiveness of L-AmB inhalations on the incidence and mortality of IA in high-risk patients with AML.

DIAGNOSIS OF CEREBRAL ASPERGILLOSIS

The diagnosis of IA is made according to the revised definitions of the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) [14]. In the revised definitions, one of the microbiological criteria is the detection of galactomannan (GM), a cell-wall component of *Aspergillus* that is released during cell growth [15]. Although the detection of GM antigen has been well validated in serum and bronchoalveolar lavage (BAL) fluid of immunocompromised patients [16,17], less is known about its diagnostic performance in cerebrospinal fluid (CSF) to diagnose cerebral aspergillosis (CA). Based on three small studies, GM antigen testing in CSF seems to be promising, even though no formal cut-off was established [18-20]. **Chapter 4** describes the results of a study that investigated the diagnostic performance of GM antigen in CSF of patients with suspected CA.

DETECTION OF AZOLE-RESISTANT *ASPERGILLUS FUMIGATUS* IN INVASIVE ASPERGILLOSIS

Over the past decade, azole resistance in *A. fumigatus* has emerged worldwide [21,22]. This is worrisome as IA with an azole-resistant *A. fumigatus* is associated with very high mortality rates of almost 90% [23,24]. Azole resistance is often caused by mutations in the *Cyp51A* gene that encodes for the lanosterol 14α-demethylase, the target enzyme for azoles. Two mutation combinations in this *Cyp51A* gene, e.g. TR_{34}/L98H and TR_{46}/T289A/Y121F, account for a large part of azole-resistant mechanisms [23,25,26] and are

believed to have developed in the environment due to azole exposure in agriculture [27,28].

Detection of azole resistance in IA is challenging for two reasons. First, a positive culture is required for conventional detection of resistance. However, cultures are positive in at most one-quarter of the cases and therefore diagnosis is often made indirectly by detection of galactomannan (GM) antigen [8,29]. Second, conventional microbiological tools for azole susceptibility testing are not widely available and time consuming. The lack of a fast and readily available azole susceptibility testing compromises the initiation of adequate therapy in case of azole resistance. Hence, there is a need for development of newer diagnostic techniques to intercept this problem.

Among the novel diagnostics are *Aspergillus* polymerase chain reaction (PCR) assays that detect and identify *Aspergillus* to the species level in different specimens [30-32]. The AsperGenius® multiplex real-time PCR is a commercially available *Aspergillus* assay. In addition to detecting *Aspergillus*, it detects the aforementioned two most common mutation combinations of *A. fumigatus* that are associated with azole resistance. Chapter 5.1 describes the results of the single-center study that investigated the diagnostic performance of this assay on BAL fluid samples of patients from the hematology department and intensive care unit. To confirm the results of the single-center study, subsequently a multicenter study was performed in a larger BAL sample set from patients with an underlying hematological disease. Moreover, we evaluated in this second study if PCR detection of the aforementioned mutation combinations correlated with azole treatment failure and mortality. The results of this multicenter study are described in Chapter 5.2.

DETECTION OF AZOLE-RESISTANT SIBLING SPECIES IN INVASIVE ASPERGILLOSIS

IA is mainly caused by *Aspergillus fumigatus*, an *Aspergillus* species from the section *Fumigati* [1,2]. Occasionally, IA is caused by *Aspergillus* species that are morphologically similar to *A. fumigatus* [33,34]. These so called 'sibling species' or 'cryptic species' also belong to the *Aspergillus* section *Fumigati* and are often intrinsically resistant to azoles. As voriconazole is the recommended first line therapy [6], fast identification and susceptibility testing of these sibling species is important to select the appropriate antifungal therapy. However, this is problematic as cultures are not available in the majority of the patients with IA [8,29]. Moreover, if a culture is available, identification and susceptibility testing is difficult as these sibling species often have poor sporulation and can only be distinguished by additional testing. **Chapter 6** describes two patients with proven IA

caused by azole-resistant sibling species and the contribution of the aforementioned AsperGenius® assay in their identification.

SUMMARY

Several studies were performed to investigate the incidence, mortality, risk factors and diagnostics of IA. **Chapter 2** focusses on the incidence, mortality and risk factors of IA in alloHSCT recipients. **Chapter 3** describes the efficacy and cost-effectiveness of L-AmB inhalations on the incidence and mortality of IA in patients with AML receiving intensive chemotherapy. The subsequent chapters focus on the diagnostics in IA. In **chapter 4**, the diagnostic performance of GM in CSF is described. **Chapter 5.1 and 5.1** present the results of two studies that evaluated the diagnostic performance of a novel PCR-assay that can not only detect *Aspergillus*, but also the two most common *Cyp51A* mutations in *A. fumigatus* that confer to azole resistance. **Chapter 6** describes the contribution of this novel PCR-assay in identifying sibling species and is followed by a general discussion in **chapter 7**.

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