

Dissertation

**Invasive Aspergillosis in Patients with Underlying
Liver Cirrhosis**

submitted by

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Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “*Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz*”.

Graz, 04.07.2017

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1 List of Abbreviations

A

ABPA = allergic bronchopulmonary aspergillosis

AIDS = acquired immune deficiency syndrome

ALT = alanine aminotransaminase

AmB = amphotericin B

AML = acute myeloid leukemia

AST = aspartate aminotransaminase

B

BALF = bronchoalveolar lavage fluid

BALF-GM = bronchoalveolar lavage fluid galactomannan

BDG = 1,3- β -D-glucan

C

CCPA = chronic cavitary pulmonary aspergillosis

CFPA = chronic fibrosing pulmonary aspergillosis

CNS = central nervous system

COPD = chronic obstructive pulmonary disease

CPA = chronic pulmonary aspergillosis

CRP = C - reactive protein

CSF = cerebrospinal fluid

CT = computed tomography

D

DNA = deoxyribonucleic acid

DM = diabetes mellitus

E

ECIL = European Conference on Infections in Leukaemia

EIA = enzyme immunoassay

EORTC/MSG = European Organization for Research and Treatment of Cancer/Mycoses Study Group

ESCMID = European Society of Clinical Microbiology and Infectious Diseases

F

FDA = United States Food and Drug Administration

G

GM = galactomannan

GVHD = graft versus host disease

H

HIV = human immunodeficiency virus

HRCT = high-resolution computed tomography

HSCT = hematopoietic stem cell transplant

I

IA = invasive aspergillosis

ICU = intensive care unit

IDSA = Infectious Diseases Society of America

IFI = invasive fungal infection

IL = interleukin

immunoPET/MR = antibody-guided positron emission tomography and magnetic resonance

ITS = internal transcribed spacer

IQR = 25 and 75 quartiles

L

LAL = Limulus Amebocyte Lysate

LFD = *Aspergillus* specific lateral-flow device

lipAmB = liposomal amphotericin B

LRT = lower respiratory tract

M

MALDI-TOF MS = matrix assisted laser desorption ionization-time of flight mass spectrometry

MHC = major histocompatibility complex

MRI = magnetic resonance imaging

N

NPV = negative predictive value

O

ODI = optical density index

P

PAMPs = pathogen-associated molecular patterns

PCR = polymerase chain reaction

PET = positron emission tomography

PMNLs = polymorphnuclear leukocytes

PPV = positive predictive value

PRRs = pathogen recognition receptors

R

ROS = reactive oxygen species

S

SAIA = subacute invasive aspergillosis

SOT = solid organ transplant

SSTI = skin and soft tissue infection

T

TAFC = triacetylfusarinine C

TDM = therapeutic drug monitoring

TLR = toll-like receptor

TNF- α = tumor necrosis factor alpha

95% CI = 95% confidence interval

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4 Abstract

4.1 Background

Invasive aspergillosis (IA) is a life threatening diseases mainly occurring in highly immunocompromised patients. Recent reports, however, raised concerns that besides those “classical” at risk patients, also patients with less severe immunosuppressive diseases may be prone to develop IA. Liver cirrhosis was mentioned as the only relevant underlying disease in several of those reports. Prospective observational data on the prevalence and outcome of IA in patients with liver cirrhosis are, however, lacking up to date. Thus, we performed a single center, prospective observational study to investigate the prevalence and outcome of IA and the performance of serum galactomannan (GM) screening in patients with underlying liver cirrhosis.

4.2 Methods

From August 2013 to December 2015, 154 consecutive patients admitted to the University Hospital of Graz, Austria with decompensated liver cirrhosis or compensated liver cirrhosis with fever and/or respiratory symptoms were enrolled in this study. All patients were screened for IA twice weekly by serum GM testing and by checking clinical signs and symptoms for IA. Positive serum GM or clinical suspicion for IA triggered work up consisting of high-resolution computed tomography of the chest and in case of pathological findings bronchoscopy and GM testing in bronchoalveolar lavage fluid. IA was classified according to slightly modified 2008 criteria of the European Organization of Research and Treatment of Cancer/Mycoses Study Group. Performance of serum GM screening was analyzed for the overall cohort as well as for patients with respiratory symptoms only and for patients with clinical suspicion of IA only.

4.3 Results

Of the 154 enrolled patients, 150 were included in the final analysis. Four patients were retrospectively excluded from the study. Reasons were: liver cirrhosis was mentioned as

underlying diseases in the emergency department but rejected after clinical work up (n=2), patient died prior to first serum GM screening (n=1), patient was included in the study within an episode of hepatic encephalopathy and refused to give informed consent after clinical improvement (n=1).

Two out of 150 patients (1.3%) were diagnosed with probable IA, one patient with possible IA (0.7%) and the remaining 147 (98%) patients had no evidence for IA. Both patients with probable IA had Child-Pugh grade A cirrhosis and were successfully treated with voriconazole under therapeutic drug monitoring and monitoring of liver function tests.

Sensitivity of serum GM screening for presence of probable IA in the overall study cohort was 0.5 [95% confidence interval (95% CI) 0.09 – 0.91], specificity 0.97 (95% CI 0.92 – 0.99), negative predictive value 0.99 (95% CI 0.96 – 0.99) and positive predictive value (PPV) 0.17 (95% CI 0.01 – 0.64). PPV was 0.5 (95% CI 0.03 – 0.98) in patients with clinical suspicion of IA.

4.4 Conclusions

Prevalence of IA in patients with underlying liver cirrhosis was low in this study. Only two patients (1.3%) were diagnosed with probable IA. These two patients, however, could be treated safely and successfully with voriconazole. Additionally, universal screening of patients with liver cirrhosis by using the serum GM antigen assay was associated with low PPVs due to low pretest probability of the disease. A more targeted approach, for example only using serum GM testing in patients with clinical suspicion for IA, seems to be more appropriate for clinical routine.

5 Zusammenfassung

5.1 Hintergrund

Die invasive Aspergillose (IA) stellt eine lebensbedrohliche Erkrankung dar, welche vor allem bei schwer immunsupprimierten Patienten/innen vorkommt. Rezente Publikationen berichten jedoch auch über das Vorkommen von IA bei Patienten/innen ohne klassische Risikofaktoren für diese Erkrankung. Bei einigen konnte als einzige relevante zugrunde liegende Erkrankung eine Leberzirrhose festgestellt werden. Prospektive Daten zur Häufigkeit der IA bei Patienten/innen mit zugrunde liegender Leberzirrhose sind bis dato jedoch nicht vorhanden. Das Ziel dieser prospektive Studie war es somit, die Häufigkeit und das Outcome der IA bei Patienten/innen mit Leberzirrhose, sowie die Performance des Serum Galaktomannan (GM) Screenings bei diesen Patienten/innen zu untersuchen.

5.2 Methoden

Von August 2013 bis Dezember 2015 wurde 154 konsekutive Patienten/innen mit zugrundeliegender dekompensierter Leberzirrhose oder kompensierter Leberzirrhose mit begleitenden Fieber und/oder respiratorischen Beschwerden welche stationär am Universitätsklinikum Graz aufgenommen wurden, in die Studie eingeschlossen. Alle Patienten/innen wurde zweimal pro Woche mittels Serum GM Tests und klinischen Kontrollen hinsichtlich des Vorliegens einer IA gescreent. Bei einem positiven Serum GM Test oder dem klinische Verdacht auf das Vorliegen einer IA wurde eine Computertomographie der Lunge durchgeführt. Im Falle von Veränderungen welche mit einer IA vereinbar waren, wurde eine Bronchoskopie mit bronchoalveolärer Lavage und eine GM Testung aus der Lavagenflüssigkeit durchgeführt. Die IA wurde anhand der 2008er Kriterien der European Organization of Research and Treatment of Cancer/Mycoses Study Group, welche für diese Studie leicht modifiziert wurden, eingeteilt. Die Performance des Serum GM Screenings in dieser Studie wurde für die gesamte Studienpopulation, sowie nur für Patienten/innen mit respiratorischen Beschwerden und zusätzlich nur für Patienten/innen mit einem bestehenden klinischen Verdacht auf das Vorliegen einer IA berechnet.

5.3 Resultate

154 Patienten/innen wurden initial in die Studie eingeschlossen und 150 davon in die finale Auswertung inkludiert. Vier Patienten/innen wurden nachträglich aus folgenden Gründen ausgeschlossen: Bei zwei Patienten/innen wurde eine zugrunde liegende Leberzirrhose in der Notaufnahme als Diagnose dokumentiert, jedoch nach weiteren Untersuchungen wieder verworfen, ein Patient verstarb kurz nach stationärer Aufnahme und noch vor dem ersten Serum GM Screening und ein Patient wurde während einer akuten Phase einer hepatischen Enzephalopathie in die Studie eingeschlossen und wollte nach klinischer Besserung nicht an der Studie teilnehmen.

Bei zwei von 150 Patienten/innen (1.3%) fand sich eine wahrscheinliche IA, bei einer Patientin (0.7%) eine mögliche IA und bei den verbleibenden 147 Patienten/innen (98%) fand sich kein Hinweis auf das Vorliegen einer IA. Beide Patienten/innen mit wahrscheinlicher IA hatten eine zugrunde liegende Child-Pugh A Leberzirrhose und konnten unter engmaschigen Voriconazol Talspiegel- und Leberfermentkontrollen erfolgreich mit Voriconazol behandelt werden.

Die Sensitivität des Serum GM Screenings in der gesamten Studienpopulation für das Vorliegen einer wahrscheinlichen IA betrug 0.5 [95% Konfidenzintervall (95% CI) 0.09 – 0.91], die Spezifität betrug 0.97 (95% CI 0.92 – 0.99), der negativ prädiktive Wert betrug 0.99 (95% CI 0.96 – 0.99) und der positiv prädiktive Wert (PPV) betrug 0.17 (95% CI 0.01 – 0.64). Der PPV betrug 0.5 (95% CI 0.03 – 0.98) für Patienten/innen mit klinischem Verdacht auf das Vorliegen einer IA.

5.4 Konklusion

Die Häufigkeit einer IA bei Patienten/innen mit Leberzirrhose war in dieser Studie gering. Nur bei zwei von 150 gescreenten Patienten/innen konnte eine wahrscheinliche IA nachgewiesen werden. Diese zwei Patienten/innen konnten jedoch erfolgreich mit Voriconazol behandelt werden. Des Weiteren, zeigte sich ein allgemeines Screening für das Vorliegen einer IA mittels des Serum GM Tests aufgrund einer geringen Vortestwahrscheinlichkeit und damit verbundenem schlechtem PPV als für die klinische

Routine nicht zweckmäßig. Der Serum GM Test sollte somit bei Patienten/innen mit Leberzirrhose nur bei vorhandenem klinischem Verdacht auf eine IA durchgeführt werden.

6 Introduction

6.1 Background

6.1.1 A Brief History of Fungal Infections

The history of fungal infections dates back to the ancient world, when Hippocrates and Galen were the first who described lesions in the human oral cavity that could probably be thrush. The association of fungi and human diseases was still far away when Giambattista Della Porta (1538-1615) was the first person who described fungal spores in 1588 ⁽¹⁾. Throughout the following decades and centuries, several human diseases were described that later were later found to be caused by fungi. Sir William Dampier (1651 – 1715) for example, described a skin disease seen in people at the Philippines that turned out as *Tinea imbricata* or “Tokelau” in the late 18th century, a superficial skin infection due to *Trichophyton concentricum* ⁽¹⁾.

The genus *Aspergillus* was first named by Pier Antonio Micheli (1679 – 1737), the founder of the Italian Botanical Society, in 1729. In his monograph “*Nova Plantarum Genera*” he described approximately 900 different fungi ⁽²⁾ and created the first drawings of *Aspergillus* species as displayed in figure 1. As one may notice, the name *Aspergillus* is based on the resemblance between a sporulating head of an *Aspergillus* species and an aspergillum, a brush which is used to sprinkle holy water. The German biologist Heinrich Friedrich Link (1767 – 1851) named the first species of *Aspergillus*, namely *Aspergillus flavus* and *Aspergillus candidus* ⁽³⁾, whilst the most prominent species among *Aspergilli*, namely *Aspergillus fumigatus*, was named by Georg Fresenius (1808-1866) in 1863 ⁽⁴⁾.

Johann Lukas Schönlein, a professor of therapeutics and pathology was the first person postulating that fungi may cause human diseases. In 1839 he described a case of *Tinea capitis favosa*, a chronic dermatophyte infection of the scalp caused by the dermatophyte *Trichophyton schoenleinii*. It is considered the first publication that describes fungi as causative microorganisms for human diseases ⁽⁵⁾. In the same year the first documented case of oropharyngeal and esophageal candidiasis found on an autopsy of a patient who died of typhus was published by Bernhard Langebeck ⁽⁶⁾, approximately 2200 years after Hippocrates described oral lesions suspected to be thrush. Only a couple of years after the first descriptions of dermatomycoses and yeast infections, it was Theodor Slyuter, a

medical student working on his doctoral thesis, who was the first person ever describing a case of pulmonary aspergillosis in humans, despite the fact that Sluyter and his colleague Johann Schauer misinterpreted the observed fungus as *Mucor mucedo* ⁽⁷⁾. Based on Slyuter's drawings, the founder of modern pathology, Robert Virchow, correctly identified the fungus later as *Aspergillus* species. It was also Virchow, who was the first to publish a detailed microscopic description of an *Aspergillus* in 1856 ⁽⁸⁾. In the following years many new *Aspergillus* species were discovered and described bringing up the need of an adequate and unique taxonomy. Charles Thom and Margaret Church published a classification of the *Aspergillus* genus in 1926 containing of 69 *Aspergillus* species classified in 11 groups ⁽⁹⁾. This, however, was only the beginning of fungal research. Thanks to new molecular diagnostic approaches, we currently know more than 250 different *Aspergillus* species classified in several different subgenera and in various sections ^(10, 11).

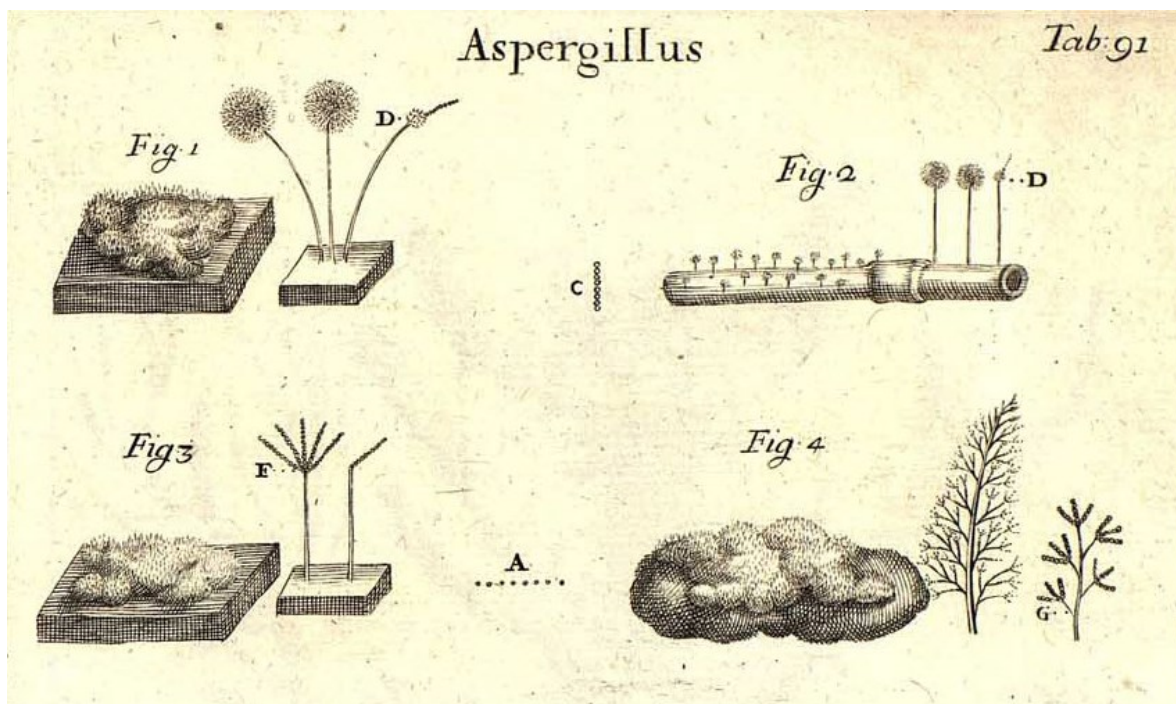


Figure 1. The first drawings from Pier Antonio Micheli of an *Aspergillus* species (Micheli, 1729)

6.1.2 The *Aspergillus* Genus

Aspergillus is an anamorph (= asexual form) fungus and member of the family Trichocomaceae. Besides the asexual form, some species within the *Aspergillus* genus are known to have a teleomorph (= sexual form) including but not limited to *Aspergillus nidulans*, *Aspergillus amstelodami* and *Aspergillus fumigatus* ^(12, 13). All of them are potential pathogens for humans. The discovery of teleomorphs, however, was accompanied by challenges for the scientific community. Correct taxonomy would have required renaming these fungi using the teleomorphs as they represent two different forms of the fungus (sexual and asexual). *Aspergillus fumigatus* for example, is named *Neosartorya fumigata* in his teleomorph. As this would have led to a very complicating and complex nomenclature systems the generic name *Aspergillus* has been retained in most cases for both states.

Nevertheless, taxonomy of *Aspergillus* has changed dramatically. This is due to new and more precise diagnostic methods, mainly molecular diagnostic approaches. Prior to molecular detection methods, *Aspergillus* species were classified by phenotype based methods. This, however, is difficult and unreliable as many different *Aspergillus* species share morphological features. Sequencing of various targets including ribosomal deoxyribonucleic acid [DNA; for example the internal transcribed spacer (ITS) region], β -tubulin or calmodulin allowed a more detailed grouping of these fungi ⁽¹⁰⁾. Based on the last classification scheme, the genus *Aspergillus* is currently classified into seven subgenera that are divided in several sections and further divided in related species ⁽¹⁴⁾.

6.1.3 Structure of the *Aspergillus* Cell

Fungi have a complex cellular structure. They contain a large amount of DNA organized in a few thousand genes. As in human cells, fungal cells are covered by a plasma membrane consisting of both lipids and proteins. Together they form a phospholipid bilayer. In contrast to human cells, however, fungal cells are stabilized by ergosterol compared to cholesterol in animal cells. The fungal cell wall is an important part of fungi as it is crucial for protection against environmental factors and also a main component for adhesion, invasion, infection, building biofilms and protection against other microorganisms. It is usually composed of a variety of polysaccharides like glucans, chitins, mannans,

galactomannans and of glycoproteins and proteins. Many of these components are found in a variety of different fungi, for example 1,3-beta-d-glucans which are nearly panfungal glucans. Other cell wall components are specific for distinct fungi or only found in a few different fungi. In *Aspergillus fumigatus*, polysaccharides represent over 90%⁽¹⁵⁾ of the cell wall components including β -1,3-glucans of which approximately 4% are branched with β -1,6-glucans, mixed β -1,3- and β -1,4-, β -1,6-, and α -1,3-glucans, chitins and galactomannans^(16, 17). Some of these components are nowadays used as biomarker for detection of invasive fungal infections (IFIs), like 1,3- β -D-glucan (BDG) or galactomannan (GM). Synthesized by various intracellular enzymes or by endoplasmic reticulum associated ribosomes, components of the cell wall are actively transported into the cell wall space where they form a three-dimensional matrix⁽¹⁷⁾. A schematic figure of an *Aspergillus fumigatus* cell wall is displayed in figure 2.

The knowledge of the structure of fungi is crucial for diagnosis and therapy of IFIs, as parts of the fungal cell are used for diagnostic purposes as already mentioned and other parts are used as targets for antifungal treatment. It is important to target structures that are essential for survival of the fungal cell on the one hand and specific for fungal cells to reduce side effects on the other hand. This is challenging as the fungal cell is similar in many ways to mammalian cells.

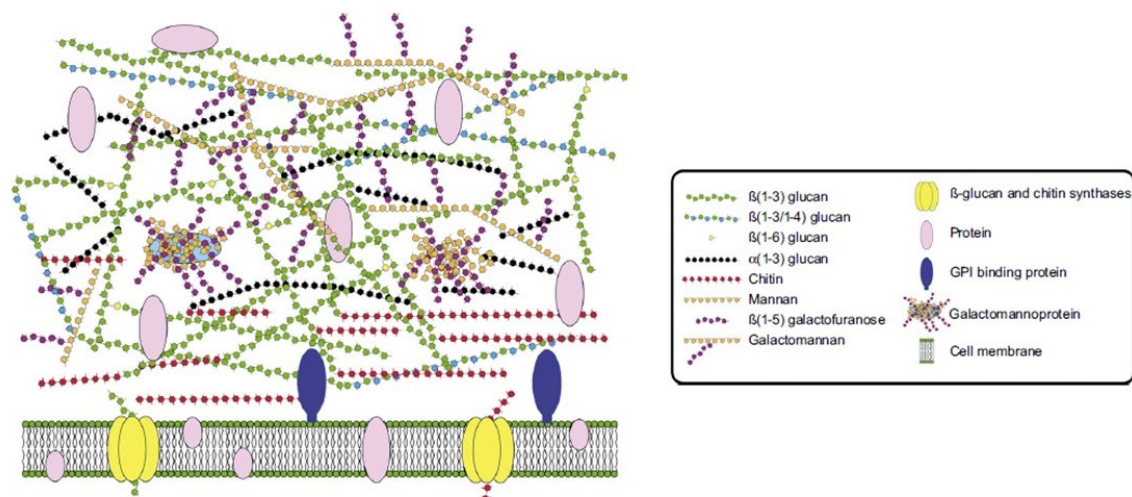


Figure 2. Schematic figure of an *Aspergillus fumigatus* cell wall⁽¹⁶⁾

6.2 Infections due to *Aspergillus*

6.2.1 Epidemiology of Invasive Aspergillosis

As *Aspergillus* species and other fungi are ubiquitous worldwide, there is a continuous exposure to these fungi. Probably a few hundred conidia are inhaled by humans every day without causing invasive disease as healthy individuals are able to handle this amount of fungal burden without developing infection⁽¹⁸⁾. In immunocompromised host the risk of developing invasive aspergillosis (IA) after exposure to *Aspergillus* is distinctively higher⁽¹⁹⁾. Between 1980 and 1997 the number of deaths due to invasive fungal diseases increased from 1,557 to 6,534 in the United States. Deaths due to IA showed a four-fold increase⁽²⁰⁾. This increase reflects the number of immunocompromised and critically ill patients and therefore the number of “at risk patients” which also highly increased within the past decades.

IA is found in a variety of immunosuppressive diseases. One of the major risk factors for IA development is prolonged and profound (<100 neutrophils/ μ L) neutropenia⁽²¹⁾. Thus, patients with acute myeloid leukemia (AML) and those undergoing hematopoietic stem cell transplant (HSCT) are at the highest risk for IA development due to the aggressive myeloablative therapy^(22, 23). Incidence of IA in AML and HSCT patients varies from study to study (see table 1). Patients receiving allogeneic HSCT are, however, at much higher risk as patients receiving autologous HSCT^(24, 25). Risk of developing IA in hematological malignancy patients does not only depend on the underlying disease. Several risk factors have been identified that are associated with the occurrence of IA in AML patients. Older age, poor performance status, diabetes mellitus (DM), chronic obstructive lung disease (COPD), jobs and hobbies accompanied with high exposure to molds (for example hunting, gardening, farming, etc.) as well as house reconstructions were significantly associated with higher risk of developing IA during chemotherapy⁽²¹⁾.

Not only hematological malignancy patients receiving cytotoxic chemotherapy are prone to develop IFIs, but also patients receiving solid organ transplants (SOT). The risk for distinct IFIs varies with the transplanted organ and the post-transplant immunosuppressive scheme. Whereas liver and pancreas transplant patients have a much higher incidence for invasive yeast infections, lung transplant recipients have the highest risk for developing IA^(26, 27). This is explained by the different types of pathogenesis of different fungi. Yeast infections

usually originate from the gastrointestinal tract, where they are considered as normal commensals within the human microbiome. In contrast, mold infections are acquired by inhalation of spores and consequently causing infections in the lungs. One year incidence rates for IA in lungs transplant recipients are reported between 2.4% – 10.1%⁽²⁶⁻²⁸⁾. Although, IA most often occurs in lung transplant recipients, liver transplant recipients have a higher mortality rate among invasive mold infections (12-week mortality rate: 27.8% versus 47.1%⁽²⁶⁾).

Other immunosuppressed patients are also at risk for IA, even though, risk is much lower in these patients than in the high risk patients. Patients with chronic lung disease (in first line COPD), patients with acquired immunodeficiency syndrome (AIDS), patients with hereditary immunodeficiency syndromes, patients with chronic granulomatous diseases and patients receiving immunosuppressive agents for other reasons, such as glucocorticoids, tumor necrosis factor- α (TNF- α) antagonist or monoclonal antibodies such as infliximab are at higher risk for development of IA⁽²⁹⁻³¹⁾. In addition, patients treated at intensive care units (ICUs) even in the absence of hematological malignancies are at risk for developing IA. In a large retrospective cohort study a prevalence for IA of 6.9% in ICU patients was reported⁽³²⁾. Interestingly, the diagnosis of IA could only be established post mortem by autopsy showing invasive growth of *Aspergillus* species in approximately 8% of the patients indicating that diagnosis of IA is challenging in this cohort⁽³²⁾. This may be due to several reasons. First, ICUs patients usually do not present with the classical clinical and radiological signs and symptoms of IA as severely immunocompromised patients do. Second, *Aspergillus* colonization in the airways is often found as a consequence of intubation, broad-spectrum antibiotic therapy, and immunosuppression. Third, differentiation among infection and colonization is often challenging. Fourth, the currently most used diagnostic criteria for IA, namely the 2008 criteria published by the European Organization of Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG)⁽³³⁾, which are used in the most studies reporting on epidemiology of IA, were designed for patients with cancer or HSCT and are not suitable for patients without such conditions. To better estimate the risk for IA in ICU patients, Meersseman and colleagues stratified ICU patients based on their underlying disease and other risk factors in three risk groups⁽³⁴⁾: high-risk, intermediate-risk and low-risk group. The high-risk group is basically restricted to hematological patients. High-risk factors are severe neutropenia, hematological malignancy and receipt of allogeneic HSCT. Intermediate-risk group consist of patients

with prolonged corticosteroid treatment prior to ICU admission, autologous HSCT, COPD, liver cirrhosis with an ICU stay >7 days, solid organ cancer, human immunodeficiency virus (HIV) infections, lung transplantation and diseases requiring long-term systemic immunosuppressive therapy. The low-risk group consists of patients with severe burns, SOT besides lung transplantation, glucocorticoid treatment ≤ 7 days, prolonged stay on ICU of >21 days, malnutrition and recent history of cardiac surgery. Besides all these different risk factors for IA development, in some patients the only relevant underlying disease was liver cirrhosis ⁽³²⁾. Liver cirrhosis as the only relevant underlying disease in patients with IA was also observed in other studies ⁽³⁵⁻⁴¹⁾. Because of their character, none of these studies was able to estimate the prevalence of IA in this cohort. Only a study on IA in patients with severe alcoholic hepatitis reported a prevalence of 15.8% for probable, proven or possible IA in these patients ⁽⁴²⁾. This high prevalence may be attributed to the underlying disease, the high prevalence of high dose glucocorticoid treatment used in these patients or a combination of both. However, the prognosis was devastating. Transplant free survival of patients with IA was 0% ⁽⁴²⁾.

Table 1. Incidence and mortality rate of invasive aspergillosis among hematological malignancy patients. Reported numbers from representative epidemiological studies.

	Cohort	Region	Cumulative IA incidence (%)	Mortality (%)	
Auberger et al.; 2008 ⁽²²⁾	Hematological malignancies	Austria	7.9	Not established	
Caira et al.; 2008 ⁽⁴³⁾	AML	Italy	10.9	Overall mortality	35
	allo HSCT		6.3		74.4
Garcia-Vidal et al.; 2008 ⁽⁴⁴⁾	allo HSCT	USA	11.5	Not established	
Koehler et al.; 2016 ⁽⁴⁵⁾	AML	Germany	6.4	12 week	33.8
	ALL		3.8		
Kontoyiannis et al. 2010 ⁽²⁴⁾	HSCT	USA	1.6	4 month	13 and 19 [#]
				1 year	13 and 36 [#]
Mariette et al.; 2016 ⁽⁴⁶⁾	ALL	France	3.3	12 week	19.2

Marr et al. 2002 (47)	allo HSCT	USA	7.3 and 10.5*	6 month	70
				1 year	80
Neofytos et al.; 2013 ⁽²⁶⁾	allo HSCT	USA	2.5	12 week	42.9
Nucci et al. 2013 (48)	AML/MDS		13.4		
	allo HSCT	Brazil	2.3	6 weeks	37
	auto HSCT		0		

*HLA matched and mismatched, respectively

#for autologous and allogeneic HSCT, respectively

Abbreviations: AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; allo HSCT = allogeneic hematopoietic stem cell transplant; auto HSCT = autologous hematopoietic stem cell transplant; ALL = acute lymphoblastic leukemia

6.2.2 Pathogenesis of Invasive Aspergillosis

IA usually originates from inhalation of *Aspergillus* conidia into the lung with consequent lung infection and potential hematogenous dissemination. Alternative but rare routes of infection are primary skin and soft tissue infections (SSTIs) or primary central nervous system (CNS) infections.

The predominant route of infection is pulmonary infection with tissue- and angioinvasion, intravascular thrombosis, tissue infarction and secondary dissemination, the hallmarks of IA (illustrated in figure 3). *Aspergillus* conidia, the infectious part of *Aspergillus* species are produced at the tip of a conidiophore, a specialized hyphae that bears conidia at their end and releases them into the air. If inhaled by humans, *Aspergillus* associated diseases may occur. Whether or not a person develops invasive disease is highly dependent on several microbial and host factors. The immune status seems to play a key role. In mouse models for example, healthy control mice were able to inhale 10^8 conidia every day without developing IA⁽⁴⁹⁾. However, in transgenic mice with induced host defense disturbances administration of only 50 conidia were enough to cause chronic inflammatory disease⁽⁵⁰⁾. After being inhaled to the human airway system, the fungus faces several defense mechanisms. Ciliary clearance for example limits access of conidia to the alveoli. Conidia size seems to play a role in case of virulence among different *Aspergillus* species. *Aspergillus fumigatus* is known to produce very small conidia with a size of only 3-5 μm , thus *Aspergillus fumigatus* conidia can penetrate deeply into the lung and have therefore an

increased pathogenicity compared to other *Aspergillus* species⁽⁵¹⁾. An additional factor for the high virulence of *Aspergillus fumigatus* compared to other *Aspergillus* species is its high tolerance to temperature differences. *Aspergillus fumigatus* grows very well at 37°Celsius but withstands high temperatures of up to 50°Celsius giving it a niche in environment⁽¹⁸⁾.

After finally reaching the alveoli, *Aspergillus* again faces several host defense mechanisms. Alveolar macrophages and epithelial cells are considered as being the most important first line defense mechanisms against invading *Aspergillus* conidia, whereas the contribution of epithelial is far less robust compared to the contribution of the macrophages. Alveolar macrophages play a key role in host defense against *Aspergillus*. They are not only responsible for phagocytosis and killing of *Aspergillus* conidia but also initiate proinflammatory responses with consequent recruitment of neutrophils to the site of infection. Neutrophils are of utmost importance as they are able to destroy conidia that evaded the primary defense by macrophages and are also able to target and kill *Aspergillus* hyphae which are too big to be phagocytosed by macrophages⁽⁵²⁾.

Macrophage activation is based on recognition of pathogen-associated molecular patterns (PAMPs) by pathogen recognition receptors (PRRs) which are expressed on the surface of macrophages. Toll-like receptor (TLR) 2, TLR 4 and dectin-1 are the most prominent and well characterized PRRs integrated in the recognition of *Aspergillus fumigatus* PAMPs⁽¹⁸⁾. After recognition and binding of these molecules, a proinflammatory cascade is induced by macrophages resulting in a production of different cytokines and chemokines like TNF- α , interleukin (IL)-1 β , IL-6, IL-8, macrophage inflammatory protein 1 α and monocyte chemoattractant protein 1⁽⁵³⁻⁵⁶⁾. While TLRs target either the conidial form, hyphal form or both, dectin-1 targets BDG⁽⁵⁷⁾. It is therefore an important PRR used by macrophages for defense against a variety of fungi, not only *Aspergillus*. Interestingly, BDG is only exposed on the cell wall of *Aspergillus* in case of active growth with swelling and germination of the conidia. In resting conidia, BDG is covered by an external cloak, a hydrophobic layer formed by the RodA protein, and therefore not accessible to dectin-1⁽⁵⁸⁾. It seems to be important for the host to only target actively and potentially invasive growing *Aspergillus* conidia as excessive inflammatory host response to resting conidia may cause more damage to host tissue than it brings benefits for the host⁽⁵²⁾. In case *Aspergillus* conidia manage to evade the primary host defense lines they may germinate

and transform from unicellular conidia to large multicellular hyphae. Thus, tissue invasion by hyphae seen at histopathology proves invasive fungal growth.

Hyphae are the primary target for polymorphonuclear leucocytes (PMNLs). Even though PMNLs also target inhaled conidia to a smaller extent ⁽⁵⁹⁾, it is the swelling of conidia that induces extensive response by PMNLs as shown in a mouse model ⁽⁶⁰⁾. PMNLs act fungicidal by releasing reactive oxygen species (ROS) ⁽⁶¹⁾ and various other antimicrobial peptides such as proteases, defensins, pentraxin-3, lysozyme, lactoferrin, and others ⁽⁶²⁾. Neutrophils are therefore considered as being critical for defense against fungal infections. This is shown in clinical studies, where prolonged neutropenia is one of the most important and relevant risk factors for developing IA ⁽⁶³⁾.

Non-neutropenic patients may also develop IA, mostly patients receiving some kind of immunosuppressive therapy, mainly glucocorticoids. Glucocorticoids affect basically every cell line involved in host defense against fungal infections. Particularly the impaired function of PMNLs under systemic glucocorticoid therapy may be one of the most important factors for increased risk for fungal infections. Adherence of PMNLs to endothelial cells with consequent migration, phagocytosis, oxidative burst, degranulation, cytokine production and chemotaxis are impaired by glucocorticoids ^(64, 65). Nevertheless, compared to neutropenic patients, IA in non-neutropenic patients tend to be less or even non-angioinvasive as there still are neutrophils that are recruited to the site of infection, compared to neutropenic patients that actually do not have any neutrophils that may limit the angioinvasive growth of *Aspergillus* ^(18, 66). Recruitment of neutrophils to lung tissue, however, also induces extensive inflammation and consequent tissue damage. This damage of lung tissue in non-neutropenic patients is suspected to be the cause of death in these patients compared to uncontrolled, systemic infection in the neutropenic patient ⁽¹⁸⁾.

Human platelets have also been shown to be able to bind to *Aspergillus fumigatus* conidia and damage them by releasing serotonin that acts fungicidal in vitro ⁽⁶⁷⁾. This may be of particular interest in patients suffering from liver cirrhosis, as these patients are often thrombocytopenic and therefore potentially more prone to development of IA compared to non-thrombocytopenic patients.

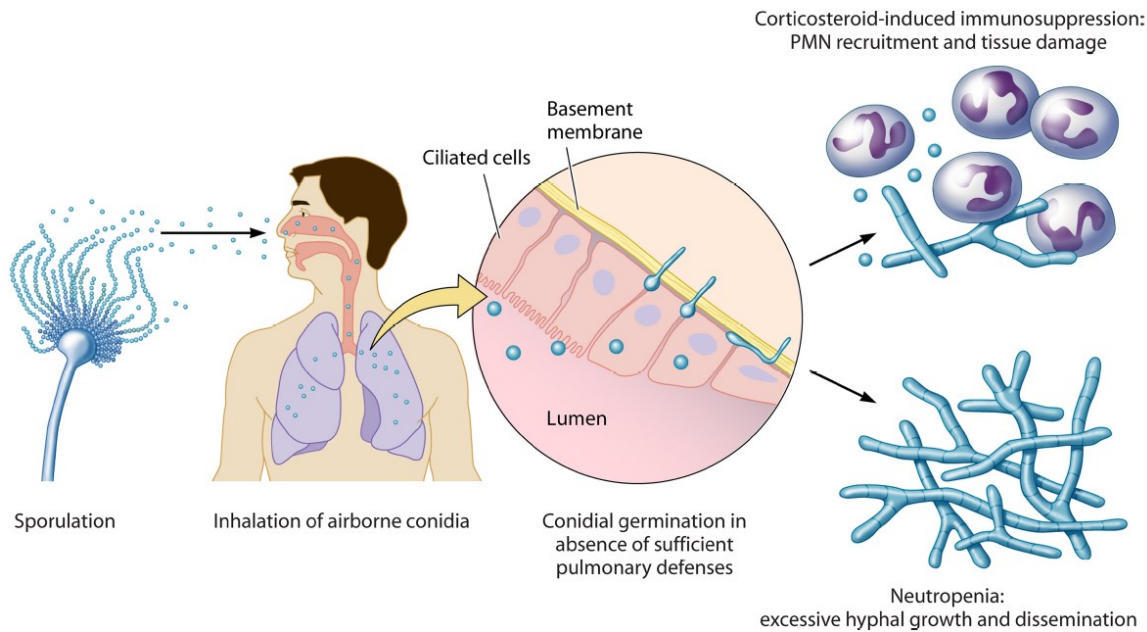


Figure 3. Schematic pathogenesis of invasive aspergillosis ⁽¹⁸⁾. After inhalation of *Aspergillus* conidia, primary defense lines (in first line alveolar macrophages and epithelial cells) are clearing the vast majority of the conidia. Some conidia may resist primary defense lines and start to germinate and invade lung tissue. In case of glucocorticoid induced immunosuppression polymorphonuclear cells are trying to clear the fungi but are limited in antifungal activity due to glucocorticoid treatment, leading to extensive inflammation and tissue damage. In case of neutropenia, hyphae may invade lung tissue and vessels, leading to uncontrolled systemic infection.

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6.2.3 Spectrum of Human Diseases Caused by *Aspergillus* Species

Aspergillus species may cause a broad spectrum of disease in human from allergic bronchopulmonary aspergillosis (ABPA) to invasive disease with systemic dissemination and sepsis syndrome. The clinical presentation of *Aspergillus* associated diseases reflects the immune status of the patient. The more severe the underlying immunodeficiency, the more invasive diseases will occur. An overview of diseases is displayed in figure 4.

ABPA is a form of *Aspergillus* associated disease that occurs in immunocompetent persons and is primarily triggered by Th2 cells ⁽⁶⁸⁾. It is considered as a hypersensitivity reaction to *Aspergillus* airway colonization and almost only found in patients with underlying pulmonary asthma or cystic fibrosis. The incidence of ABPA in asthma patients is approximately 1% to 2% and 7% (reported range of 2% to 15%) in cystic fibrosis patients ⁽⁵¹⁾. ABPA may present clinically by deterioration of pulmonary function, exacerbation of asthma, wheezing, pulmonary infiltrates due to atelectasis, central bronchiectasis or fibrosis. ABPA should be suspected in any of the mentioned syndromes in cystic fibrosis patients, as it typically progresses through several exacerbations and remissions and may

lead to early pulmonary fibrosis. Fibrosis is associated with disease progression and bad prognosis. Diagnosis, however, remains difficult as various serological markers, for example elevated blood eosinophils are unspecific and may be found in other diseases causing similar symptoms. Strategies for screening and diagnosis for asthmatic and cystic fibrosis patients have been published for early recognition of ABPA using clinical signs and symptoms, serological testing and radiological findings ^(51, 69). Treatment targets reduction of asthmatic symptoms and limitation of progression of lung fibrosis. Systemic glucocorticoids are first line agents by using 0.5 – 2.0 mg/kg/day prednisolone for 1-2 weeks with consecutive tapering. The role of antifungals is inconclusive. However, if there is no or only poor response to systemic glucocorticoids, oral itraconazole should be considered ⁽⁵¹⁾. In a placebo controlled trial, itraconazole 200 mg per day could significantly reduce the use of glucocorticoids within 16 weeks of follow up and improved pulmonary function ⁽⁷⁰⁾.

Recently, comprehensive guidelines for diagnosis and management of chronic pulmonary aspergillosis (CPA) were published ⁽⁷¹⁾. CPA consists of several clinical and radiological manifestations: simple pulmonary aspergilloma, *Aspergillus* nodule(s), chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA) and subacute invasive aspergillosis (SAIA). All those forms of CPA are primarily observed in non-immunocompromised hosts with some kind of prior underlying pulmonary disease, like COPD, tuberculosis or others. Disease duration of three month is used to distinguish between chronic forms of aspergillosis and acute or subacute forms of aspergillosis. The most common form of CPA is CCPA that may progress to CFPA if untreated leading to major loss of healthy lung tissue, lung function and consecutively loss of living quality. SAIA is a newly introduced term (formally known as chronic necrotising pulmonary aspergillosis) and usually associated with mild degree of immunosuppression. It typically occurs in patients with diabetes mellitus, malnutrition, alcoholism, advanced age, prolonged use of glucocorticoids or other slightly immunocompromising drugs, COPD, connective tissue disorders, radiation therapy, nontuberculous mycobacterial infection, or HIV infections ⁽⁷¹⁾. In contrast to chronic non-invasive forms, SAIA may be associated with GM antigenemia and detectable GM in blood ⁽⁷²⁾. Diagnosis of CPA consists of a synopsis of clinical findings, radiological signs [usually chest computed tomography (CT)], direct evidence of *Aspergillus* infection [for example positive culture or positive polymerase chain reaction (PCR)] or immunological response (serology) and exclusion of

differential diagnoses⁽⁷¹⁾. Treatment strategies depend on existing form of CPA. While in case of a simple aspergilloma surgical resection without systemic antifungal treatment is appropriate, other forms of CPA require systemic antifungal treatment with either itraconazole or voriconazole for a prolonged period of time (minimum of 4-6 months)⁽⁷¹⁾.

IA is the most severe form of aspergillosis accompanied with high mortality rates. Between 1980s and 1990s there was a significant increase in mortality due to *Aspergillus* associated disease⁽²⁰⁾ but mortality rates declined afterwards in the area of mold active prophylaxis in high risk patients. Currently, mortality rates of approximately 25% are reported in hematological malignancy patients⁽⁷³⁾ but may also reach 100% in patients with severe alcoholic hepatitis⁽⁴²⁾. Pathogenesis and epidemiology of IA are described above. Diagnosis and treatment are described below.

Rare manifestations of *Aspergillus* infections are primary SSTIs or CNS infections. SSTIs may occur as consequence of disruption of the integrity of the skin barrier (e.g. after surgical interventions, burns or a trauma), due to contaminated intravenous catheters or after laser tattoo removals⁽⁷⁴⁻⁷⁶⁾. Localized SSTIs due to *Aspergillus* species have a very good prognosis compared to IA but they may disseminate in the immunocompromised host, similar to pulmonary infections, leading to similar prognosis as for disseminated pulmonary IA⁽⁷⁶⁾. CNS aspergillosis is a very rare condition. In fact, only 5-10% of all CNS fungal infections are caused by *Aspergillus* species^(77, 78). However, prognosis of CNS aspergillosis is very bad. Of all forms of *Aspergillus* infections, CNS aspergillosis is accompanied with the highest mortality rate⁽⁷⁹⁾. Occurrence of CNS infection is usually secondary after dissemination of a primarily infected site, mostly lungs or paranasal sinuses. Direct infections after head trauma or neurosurgery, however, are also reported⁽⁸⁰⁾. In some cases none of the previously mentioned routes could have been established and are therefore considered as primary CNS infections⁽⁸¹⁻⁸⁴⁾. Most of these patients had some kind of underlying immunosuppressive disease, such as AIDS.

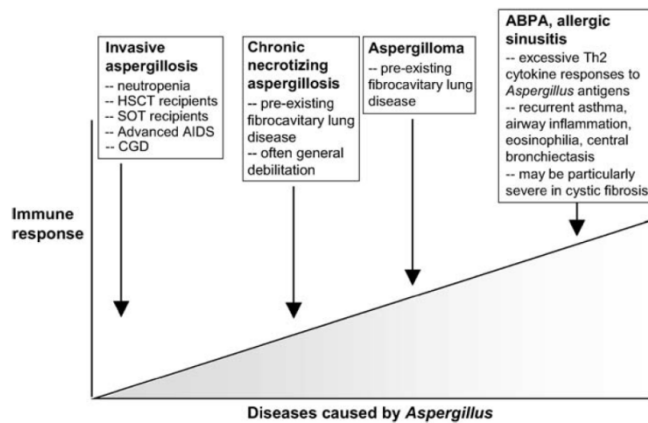


Figure 4. Overview of diseases caused by *Aspergillus* species in humans ⁽⁸⁵⁾. Allergic bronchopulmonary asthma (ABPA) is observed in atopic patients with no or only very mild underlying immunosuppressive diseases.

Aspergilloma and chronic necrotizing aspergillosis (now renamed to subacute invasive aspergillosis) are forms of chronic pulmonary aspergillosis. These diseases are observed in modest immunosuppressed patients and usually localized in the lungs without systemic spreading. Invasive aspergillosis is the most severe form of *Aspergillus* diseases and observed in severely immunocompromised patients. It is associated with invasive growth, systemic spreading and high mortality rates.

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6.3 Diagnosis of Invasive Aspergillosis

The critical step for successful management of IA is rapid initiation of antifungal therapy. This, however, implicates early and reliable diagnosis of IA in all different cohorts of patients from the severely immunosuppressed allogeneic HSCT patient to not or only modest immunosuppressed patient with for example COPD. The importance of early initiation of antifungal treatment is well described for candidemia. Mortality rates of these patients are closely associated with time of initiation of treatment. With immediate initiation of therapy mortality rate in candidemic patients reaches 15% whereas it increases with a delay of 1, 2 or ≥ 3 days to 24%, 37% and 41% in a representative study of 230 patients with candidemia ⁽⁸⁶⁾. Similar observations are reported for IA ^(87, 88). Very impressive is the devastating high mortality rate of >90% in case therapy for IA is withheld for more than 10 days compared to 41% with early start of antifungals ⁽⁸⁸⁾. For a long period of time, culture was the only diagnostic approach for IA. Fungal culture, however, lacks sensitivity, comes with a long turn around and may only indicate colonization rather than real infection ⁽⁸⁹⁻⁹¹⁾. Therefore, diagnostic tools based on fungal antigen detection and molecular based diagnostic tools have been developed within the last decade and have demonstrated their potential in IA diagnosis ^(92, 93). Diagnosis of IA, however, requires not only positive culture or biomarkers but is based on synopsis of risk factors for IA development, clinical presentation, imaging studies, histopathology (if available) and microbiology. This is well reflected in the currently available diagnostic criteria for IA published in 2008 by the European Organization of Research and Treatment of

Cancer/Mycoses Study Group (EORTC/MSG) ⁽³³⁾ categorizing IFIs into proven, probable and possible IFIs (table 2). Currently, a revision of these definitions is on its way.

Table 2. Diagnostic criteria for invasive aspergillosis according to the 2008 revised definition from the European Organization of Research and Treatment of Cancer/Mycoses Study Group ⁽³³⁾

Proven invasive aspergillosis (one of the following points must be met)

Microscopic analysis on sterile material

Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage

Culture on sterile material

Recovery of *Aspergillus* by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BALF, a cranial sinus cavity specimen, and urine.

Recovery of *Aspergillus* by blood culture

Probable invasive aspergillosis (host, clinical and microbiological criteria must be met)

Host criteria (one of the following must be met)

- Recent history of neutropenia ($<0.5 \times 10^9$ neutrophils/L) for >10 days
- Receipt of an allogeneic stem cell transplant
- Prolonged use of corticosteroids at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks
- Treatment with other T-cell immunosuppressants such as cyclosporine, TNF- α blockers, specific monoclonal antibodies, or nucleoside analogues during the past 90 days
- Inherited severe immunodeficiency

Clinical criteria (one of the following must be met)

Lower respiratory tract fungal disease

The presence of at least 1 of the following 3 signs on CT scans:

- Dense, well-circumscribed lesions(s) with or without a halo sign
- Air-crescent sign
- Cavity

Tracheobronchitis

Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopy

Sinonasal infection

Imaging showing sinusitis plus at least 1 of the following 3 signs:

- Acute localized pain (including pain radiating to the eye)
- Nasal ulcer with black eschar
- Extension from the paranasal sinus across bony barriers, including into the orbit

Central nervous system infections

1 of the following 2 signs:

- Focal lesions on imaging
- Meningeal enhancement on MRI or CT

Mycological criteria (one of the following must be met)

- Direct test (cytology, direct microscopy, or culture) on sputum, BALF, bronchial brush or sinus aspirate indicating presence of fungal elements or culture recovery *Aspergillus* spp.
- Indirect tests (detection of antigen or cell-wall constituents): Galactomannan antigen detected in plasma, serum, BALF, or CSF

Possible invasive aspergillosis

Presence of host criteria and clinical criteria but absence of mycological criteria

Abbreviations: BALF = bronchoalveolar lavage fluid; CSF = cerebrospinal fluid; CT = computed tomography; MRI = magnetic resonance imaging; TNF- α = tumor necrosis factor alpha

6.3.1 Aspergillus Culture

For long time culturing specimens from patients with suspected *Aspergillus* infections was the only microbiological approach for diagnosis of IA. Culture is recommended whenever possible ⁽⁹⁴⁾ as it is a simple and cheap diagnostic tool, it allows antifungal susceptible testing and is an important tool for surveillance of epidemiological purposes. In accordance with suitable clinical symptoms and radiological findings consistent with IA, a positive lower respiratory tract (LRT) culture for *Aspergillus* is sufficient to diagnose probable IA based on the EORTC/MSG criteria ⁽³³⁾. It, however, has to be mentioned that these diagnostic definitions were designed for research purposes only. Thus, positive cultures in

respiratory samples for *Aspergillus* can only support the diagnosis of IA in clinical routine. Positive blood cultures for *Aspergillus* rarely occur. Even in case of widely disseminated disease, blood culture usually remain negative⁽⁹⁵⁾. If it occurs, aspergillemia usually occurs late in the course of disease and is exclusively seen in patients with underlying hematological malignancies⁽⁹⁶⁾. Similar limitations are observed for bronchoalveolar lavage fluid (BALF) cultures obtained by bronchoscopy. Depending on the examined patients' cohort BALF culture yield sensitivities of 19% - 50% only^(35, 91, 97-100). Sensitivity may be even lower in case of systemic antifungal prophylaxis or therapy⁽¹⁰¹⁾. Positive BALF cultures for *Aspergillus* always have to be interpreted in context with risk factors for IA as the positive predictive value (PPV) of a positive culture increases with the degree of immunosuppression on the one hand but positive culture may also indicate colonization only instead of real infection^(102, 103).

After culturing, *Aspergillus* has to be identified to the species level. Method of choice for identification of cultured fungi is still microscopy and macroscopic examination. New molecular based methods and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), however, have shown promising results for fast and reliable identification^(10, 104).

6.3.2 Histopathology and Cytology

Direct examination should be performed for all clinical samples obtained from patients with suspected IFIs⁽⁹⁴⁾. This is of importance, as proven diagnosis of IA requires tissue samples showing invasive growth of *Aspergillus* hyphae or alternatively positive culture from an normal sterile side, for example blood or cerebrospinal fluid (CSF)⁽³³⁾. Histopathology may also allow distinguishing between *Aspergillus* colonization, in case *Aspergillus* was found in LTR culture, and true invasive infection. Even though histopathology is the current gold standard for diagnosis of proven IA, its clinical applicability is limited. First, patients at risk for developing IA are also those who are at the highest risk for developing complications that may occur within sampling procedures, like tissue biopsy^(105, 106). For example, patients with chemotherapy induced neutropenia are also very likely to have thrombocytopenia that increases the risk of bleeding with invasive procedures such as CT guided puncture^(106, 107). Second, even though presumptive diagnosis (e.g. *Aspergillus* species or other molds) can be attempted due to their

morphological characteristics leading to fast diagnosis, sensitivity and specificity are limited ⁽⁹⁰⁾. To maximize diagnostic value of histopathology and cytology adequate quantity of tissue, short turn-around times, incubation of the sample for at least five days and communication of suspicion of fungal infection from the clinician to the laboratory are recommended ⁽¹⁰⁸⁾. In case of tissue biopsy and presence of *Aspergillus*, the fungal hyphae can easily be seen with common staining methods such as Gomori methenamine silver or periodic acid-Schiff. It, however, has to be mentioned, that even though most molds have a distinct morphology definitive diagnose of the pathogen requires identification of the fungus by culture or non-cultural based methods ⁽⁹⁴⁾.

6.3.3 Imaging Studies

6.3.3.1 Computed Tomography

High-resolution CT scan (HRCT) of the chest is recommended whenever IA is suspected regardless of chest radiography results ⁽⁹⁴⁾. It is superior to chest radiography as it may also detect small nodules and typical signs of IA ⁽¹⁰⁹⁾. In addition to information on the potential extent of disease HRCT is also an important tool to guide further diagnostic actions such as CT-guided puncture or bronchoscopy and BALF sampling ⁽¹¹⁰⁾. In hematological malignancy patients early HRCT scan in case of new onset of fever during chemotherapy has been shown to increase survival among these patients as it may distinguish different reasons for pulmonary deterioration or fever (bacterial or fungal pneumonia) and is therefore helpful for empiric therapy ^(87, 111, 112). Typical features of IA in CT imaging are dense, well-circumscribed lesion(s) with or without halo sign, air-crescent sign and cavity lesions ⁽³³⁾. Examples are displayed in figure 5.

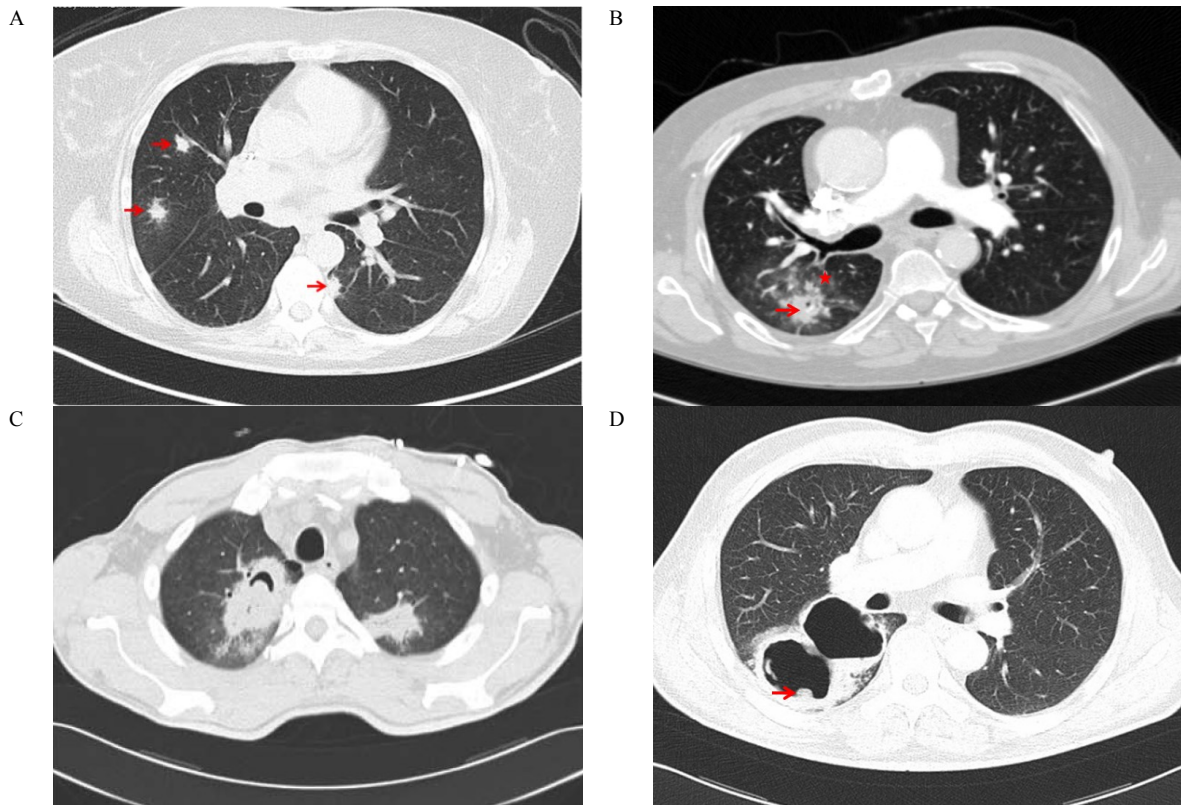


Figure 5. Features of pulmonary *Aspergillus* infections in computed tomography scans. A) Three macronodules ≥ 1 cm in diameter (red arrows) in a patient with probable IA after autologous stem cell transplantation. B.) A hyperdense lesion (red arrow) with a surrounding halo sign (red star) in a 49 year old heart transplant recipient with probable IA. C.) Air crescent sign which is usually observed in patients with IA after recovery of neutropenia (obtained from ⁽¹¹³⁾). D.) An aspergilloma consisting of a large cavity with a fungus ball inside (red arrow) in a 78 year old male patient.

The halo sign, considered a classical sign highly suspicious for presence of IA, presents as pulmonary nodule with surrounding ground-glass opacity. It is caused by pulmonary infarction due to *Aspergillus* and surrounding alveolar hemorrhage. As this type of CT finding requires angio-invasion it is typically found in patients with severe neutropenia and usually presents in the early course of disease (within the first two weeks) ^(87, 114-117). Air crescent sign, a crescent-shaped area of radiolucency within a parenchymal consolidation or nodule, usually follows the halo sign after recovery from neutropenia. The most common finding on chest CT scans in IA, however, are nodules ≥ 1 cm in diameter, observed in nearly 95% of patients ⁽¹¹⁶⁾. Many of the described lesions may also appear in other diseases, like rheumatological diseases. CT angiography may help to discriminate IA from other causes of lung nodules as in contrast to other pulmonary pathogens *Aspergillus* causes angio-invasion and consequent vessel occlusion in neutropenic patients that can be observed in CT angiography ^(118, 119). In a study published by Marta Stanzani 2015 ⁽¹¹⁹⁾, a missing vessel occlusion could rule out invasive mold diseases in 100% of patients.

Implementing these findings in clinical routine, CT angiography may be used in high risk patients and in case of missing vessel occlusion empiric antifungal therapy may be discontinued or withheld.

Serial CT scans may also be used for follow up and mortality prediction. This was recently shown in study from Leuven/Belgium ⁽¹²⁰⁾ which assessed the dynamics in CT findings in an animal model. Even though many animals had to be killed after a few days due to ethical reasons, animals with a high burden of infection showed more intense CT findings compared to animals with less progression of diseases that also correlated to dynamics in investigated biomarkers. However, in the first two days after infection there was no difference in CT scores between infected and control mice, indicating that CT scans in patients cannot rule out IA in case they are performed very early in the course of disease. For clinical routine the 2016 *Aspergillus* guidelines by the Infectious Diseases Society of America (IDSA) ⁽⁹⁴⁾ recommend a follow up CT scan not before 2 weeks of start of treatment, as pulmonary lesions can increase up to 4-fold within the first week before becoming stable ⁽¹¹⁵⁾. A recently published study ⁽¹²¹⁾, however, questions this recommendation as they found that any progression in CT scans between day 7 and they 14 correlated with a bad outcome. CT scans after day 14, however, had low additional value regarding outcome. These findings may indicate that early follow up (within a week) and consequent adjustment of therapy may be of benefit for patients with increasing lung nodules.

6.3.3.2 Magnetic Resonance Imaging

The role of magnetic resonance imaging (MRI) for IA is limited and has no or only limited additional value compared to chest HRCT ⁽¹²²⁾. MRI is therefore not recommended for routine use ⁽⁹⁴⁾ as it is technically more sophisticated, takes longer time and is more expensive. Additionally, in contrast to HRCT findings in MRI suspected for IA are not well described or defined. Advantages of MRI that may justify clinical use in selected cases are that MRI is a radiation free procedure and the use of contrast agents with fewer side effects compared to CT contrast agents which may be used in special circumstances (e.g. CT angiography). Regarding bone infections, paranasal sinus infections, or cerebral infections, MRI is the imaging study of choice ^(94, 123, 124).

6.3.3.3 New Imaging Approaches

There are some new kids on the block that may allow earlier and more specific diagnosis of pulmonary *Aspergillus* infections. Antibody-guided positron emission tomography and magnetic resonance (immunoPET/MR) has recently shown promising results for diagnosis of pulmonary IA ⁽¹²⁵⁾. An *Aspergillus* specific monoclonal antibody (JF5) was radiolabeled with [⁶⁴Cu]DOTA and able to distinguish between *Aspergillus* caused pneumonia and bacterial pneumonia in an animal model (figure 6) in contrast to conventional [¹⁸F]FDG positron emission tomography (PET) scans. A similar approach was followed by a group from Innsbruck/Austria ⁽¹²⁶⁾. They used ⁶⁸Ga labeled triacetylfusarinine C (TAFC), an *Aspergillus fumigatus* specific siderophore, for PET studies in mice. ⁶⁸Ga TAFC showed rapid accumulation in the lungs of infected animals and the amount of accumulation correlated well with the severity of disease ⁽¹²⁶⁾.

Both approaches use highly specific tracers for *Aspergillus* infections and may be used in future for discriminating *Aspergillus* from non-*Aspergillus* caused pulmonary infections in patients at risk for developing pulmonary *Aspergillus* infections.

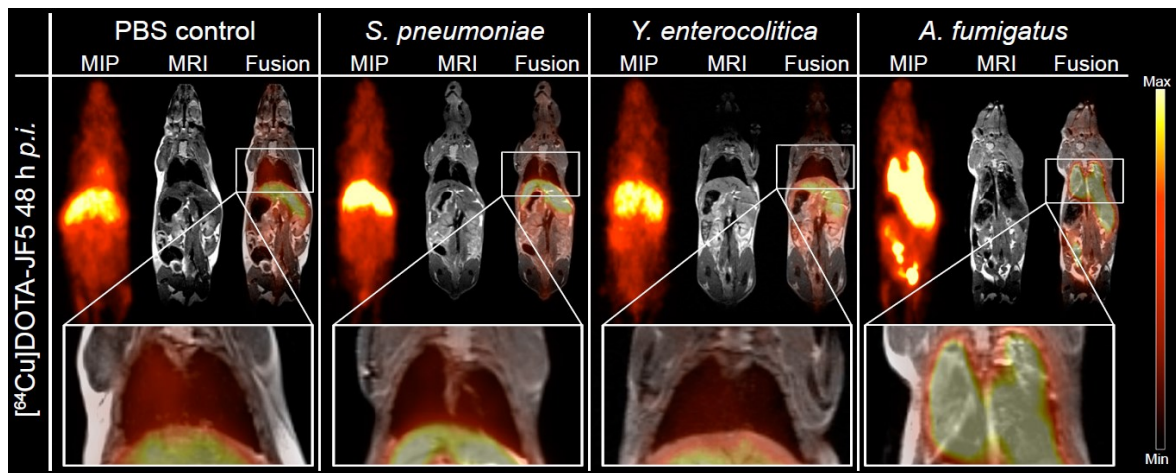


Figure 6. Sagittal maximum intensity projections (MIP), MRI, and fused PET/MRI scans in animals 48h after infection ⁽¹²⁵⁾. [⁶⁴Cu]DOTA-JF5 tracer was highly specific for *Aspergillus fumigatus* infections. In control mice and mice infected with *Streptococcus pneumoniae* or *Yersinia enterocolitica*, no [⁶⁴Cu]DOTA-JF5 tracer uptake could be observed. Copyright © 2017 PNAS.

6.3.4 Biomarkers for Invasive Aspergillosis

6.3.4.1 Galactomannan

Galactomannan (GM) is a circulating polysaccharide cell wall component of *Aspergillus* species that is released into the bloodstream and surrounding tissue by growing hyphae and

germinating conidia in case of tissue invasion. GM may be detected in several body fluids including blood⁽¹²⁷⁾, BALF^(91, 99, 128), urine⁽¹²⁹⁾ or CSF⁽¹³⁰⁾ by using a United States Food and Drug Administration (FDA) approved double-sandwich enzyme immunoassay [EIA (Platelia *Aspergillus* EIA; Bio-Rad Laboratories, Inc.)]. In detail, the GM EIA uses the rat EBA-2 monoclonal antibody directed against *Aspergillus* galactomannan. Cross reactions with GM from other fungal species may be observed for *Fusarium* species⁽¹³¹⁾, *Penicillium* species⁽¹³²⁾, *Histoplasma* species⁽¹³³⁾ or *Geosmithia argillacea*⁽¹³⁴⁾. False positive GM results have been published in case of use of GM containing blood products⁽¹³⁵⁾, use of plasmalyte⁽¹³⁶⁾, antibiotics and food products⁽¹³⁷⁾. Piperacillin/tazobactam administration was also suspected to cause false positive serum GM levels⁽¹³⁸⁾. However, this was not observed anymore after the introduction of a new formulation and probably due to GM contamination in only a few batches^(139, 140). False negative results occur due to preparation of viscous BALF samples with dithiothreitol-based mucolytic agents^(141, 142), antifungal therapy, encapsulated infection or low fungal burden^(19, 143). Results of this assay are reported as optical density index (ODI) which is the index of the optical density of the patients samples divided by the optical density of a positive control.

FDA approved cut-off for positivity is ≥ 0.5 ODI. Optimal cut-off, however, strongly depends on patient characteristics and tested samples. For serum samples in leukemic patients and HSCT patients a cut-off for positivity of 0.5 is recommended⁽¹⁴⁴⁾. Higher cut-offs (1.0 and 1.5 ODI) have been evaluated but showed distinctively lower sensitivities by only slightly higher specificities limiting their use in a screening test⁽¹⁴⁵⁾. The optimal cut-off for non-neutropenic patients, however, is still a matter of debate. In general, it seems as serum GM determination in non-neutropenic patients yield much lower sensitivity compared to neutropenic patients. Two meta-analyses including approximately 4,000 patients yielded a sensitivity of 71% - 78% and a specificity of 81% - 89% for serum GM testing for diagnosis proven IA in immunocompromised patients^(127, 146). Subgroup analysis revealed better serum GM performance for patients receiving HSCT or with underlying hematological malignancy compared to solid organ recipients⁽¹⁴⁶⁾. Whether the more aggressive angio-invasive growth⁽⁶⁶⁾ and consequent antigenemia in neutropenic patients or the lower fungal burden in non-neutropenic patients may cause this difference in serum GM performance remains to be clarified. A fairly recently published study, however, confirmed the results from this meta-analysis. In a non-neutropenic cohort, the sensitivity of serum GM testing was 38% only⁽¹⁴⁷⁾ which is in accordance with other published studies^(32, 148, 149). Even though in neutropenic patients, performance of serum

GM detection is significantly lower in case of ongoing systemic antifungal prophylaxis or therapy⁽¹⁵⁰⁻¹⁵²⁾. Performance of serum GM testing in patients with underlying liver cirrhosis, however, is completely unknown.

Serum GM represents an elegant and only minimal invasive approach for IA screening and diagnosis. Considering that IA usually originates from lung infections, GM determination in BALF is the current gold standard for IA diagnosis, however, obtaining BALF requires bronchoscopy and is therefore a far more invasive procedure. Performance of BALF-GM in two meta-analyses on patients with different underlying diseases revealed a pooled sensitivity of 85% - 90% and a pooled specificity of 90% - 95%^(128, 153). The debate about the optimal cut-off in BALF samples is still ongoing. Usually higher levels compared to serum (0.7 – 1.0) are used as a cut-off for positivity. Levels <0.5 and ≥ 3 are associated with very high negative predictive value (NPV) and PPV (96.6% and 100% respectively) and may therefore be of most value for clinicians to either rule out or diagnose IA⁽¹⁵⁴⁾. BALF-GM determination is also more robust against antifungal prophylaxis or therapy compared to serum GM⁽¹⁵⁵⁾. However, also BALF-GM sensitivity may be decreased in case of systemic antifungal therapy⁽¹⁰¹⁾. BALF-GM in non-neutropenic patients yield nearly the same sensitivities and specificities than in neutropenic patients^(91, 98, 147, 156, 157), indicating that in patient without neutropenia and a negative serum GM but clinical and radiological findings consistent with presence of IA, bronchoscopy and BALF-GM should be performed.

6.3.4.2 1,3- β -D-Glucan

1,3- β -D-glucan (BDG) is a fungal cell wall component of most pathogenic fungi with exception of Mucorales, that are not known to produce BDG, and *Cryptococcus* species, that are known to produce only very low amounts of BDG^(158, 159). Thus, BDG testing is not specific for *Aspergillus* infections and is considered a (nearly) panfungal biomarker. Several different BDG tests using different methods and substrates are available. Performance may vary significantly within the different tests⁽¹⁴⁴⁾. Only the Fungitell[®] assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA) is currently FDA approved. It is based on the Limulus Amebocyte Lysate (LAL) assay. The LAL assay may be activated by either endotoxins leading to activation of Factor C or by BDG leading to activation of Factor G. Factor C, however, is inactivated in the Fungitell[®] assay to guarantee BDG specificity (figure 7). Factor G, isolated from the North American

Horseshoe Crab, activates a coagulation cascade when getting into contact with BDG that is used in this colorimetric assay. Cut-offs for negativity and positivity for the Fungitell[®] assay recommended by the manufacturer are <60 pg/mL and ≥80 pg/mL respectively. In general, the performance of serum BDG testing for IFIs is very promising in terms of sensitivity, specificity and most of all in terms of NPV. A recent meta-analysis of approximately 600 proven IFIs (the majority of patients had *Candida* species infections and patients with *Pneumocystis jirovecii* infections were excluded) revealed a pooled sensitivity of 77% and specificity of 85%⁽¹⁶⁰⁾. In another meta-analysis by Lamoth et al.⁽¹⁶¹⁾ an overall PPV of approximately 84% and an excellent NPV of approximately 95% was determined emphasizing the remarkable potential of this BDG assay for IFI diagnosis. Other studies reported even NPVs up to 100%⁽¹⁶²⁾. To increase specificity studies have investigated the role of two or more positive consecutive BDG test required for IFI diagnosis. In general specificity is increased if two or more positive tests are required for diagnosis, however, sensitivity decreases, probably even significantly⁽¹⁶²⁾. Of interest is that three consecutive positive serum BDG yield of up to 100% PPV for presence of IFI^(162, 163). High NPV of BDG assays may help to direct empiric antifungal therapy. We could show, that negative serum BDG levels are a safe method to discontinue empiric antifungal therapy in ICU patients⁽¹⁶⁴⁾. This strategy significantly reduces use of antifungals in patients without IFIs and therefore helps to reduce costs. In a study from Italy⁽¹⁶⁵⁾ using BDG as guidance for antifungal treatment in ICU patients, the average cost reduction regarding antifungal therapy was €3540 per each patient with reduced, discontinued or withheld antifungal therapy. Importantly, only one of the patients without antifungal therapy developed candidaemia.

The diagnostic performance for IA is usually limited due to the nearly panfungal presence of BDG. In detail specificity reaches between 74% and 88%⁽¹⁶⁶⁻¹⁶⁹⁾. However, in high risk patients receiving mold active prophylaxis a recently published study suggests a very promising performance of serum BDG screening for IA⁽¹⁶³⁾. BDG determination in BALF is not suitable for diagnosis of IFIs^(170, 171) as *Candida* belongs to the normal human lung microbiome and is frequently found in patients, especially in ICU patients^(172, 173). As *Candida* does not cause pneumonia⁽¹⁷⁴⁾, BDG in BALF may only detected the presence of *Candida* and therefore has a very low specificity when determined in BALF. BALF-BDG levels, however, may be of prognostic value⁽¹⁷⁵⁾.

Positive serum BDG results always have to be interpreted with caution, as there are several factors known to cause falsely elevated BDG levels like administration of blood products, broad-spectrum antibiotics, severe mucositis, *Enterococcus faecalis* bacteremia or major surgeries⁽¹⁷⁶⁻¹⁸¹⁾. Hemodialysis was also suspected to cause false positive serum BDG levels⁽¹⁸²⁾. This, however, was due to the use of cellulose containing membranes that are not used anymore. Thus, hemodialysis nowadays does not cause elevated serum BDG levels anymore⁽¹⁸³⁾.

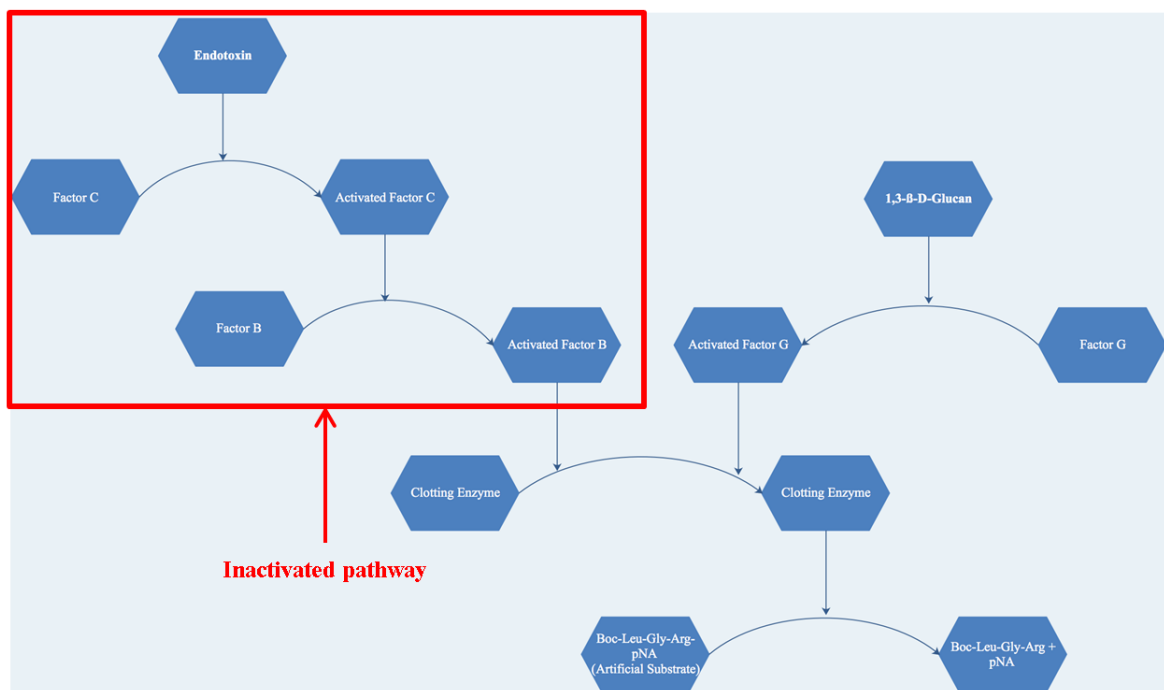


Figure 7. Principle of the Fungitell® 1,3-β-D-glucan assay. Whilst the Limulus Amebocyte Lysate assay may be activated by either BDG or endotoxins, the endotoxin pathway is inactivated in the Fungitell assay to guarantee BDG specificity.

6.3.4.3 New Biomarkers

Neither GM nor BDG are perfect biomarkers for diagnosis of IA and coming along with several limitations. Thus, new diagnostic approaches have continuously been investigated.

Triacetylfusarinine C, which has been evaluated as radiolabeled biomarker in imaging studies, may also be of diagnostic value in BALF. Especially when combined with BALF-GM testing it seems to be a promising diagnostic approach ⁽¹⁸⁴⁾.

A new diagnostic tool is the *Aspergillus* specific lateral-flow device (LFD) assay. The LFD is an immuno-chromatographic point-of-care test that detects an extracellular mannoprotein secreted exclusively during active growth of *Aspergillus* species ⁽¹⁸⁵⁾. Compared to the GM assay, the LFD provides some potential advantages ⁽¹⁸⁶⁾. The LFD may be easily performed without a special equipped laboratory or specially trained staff, it yields rapid results (within 15 minutes) and the monoclonal JF5 antibody used for antigen detection cross reacts with antigens from certain *Penicillium* species only and not with a number of other pathogenic fungi detected by the GM-EIA ^(185, 187, 188). In addition, as shown in an animal model of IPA, performance of the BALF LFD was not strongly influenced by systemic antifungals and is reproducible along different laboratories, which is important as results are read by naked eye (figure 8) ^(189, 190). Sensitivity of BALF-LFD reaches between 71% and 100% and specificity between 76% and 95% for various patient cohorts ^(35, 91, 98, 186, 191, 192).

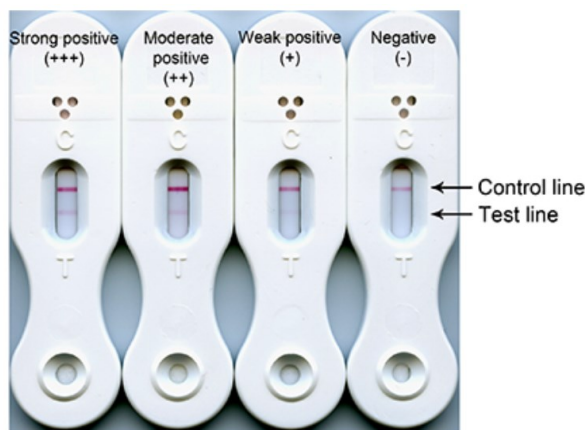


Figure 8. *Aspergillus* specific lateral-flow assay ⁽¹⁸⁸⁾. A negative control (-) and three positive controls (+, ++ and +++) are displayed. The LFD test distinguishes between weak (+) and strong (+++) positive results according to the intensity of the test line. All positive BALF-LFD results are indicating germination of spores, regardless of the intensity of the test line, and are therefore suspicious for presence of invasive aspergillosis.

Recently a new “super-antigen”, a dihexasaccharide, was shown to be the first antigen that can be found in *Aspergillus* infections, *Candida* infections and in Mucormycoses ⁽¹⁹³⁾. Performance was best for *Aspergillus* infections but detection of this antigen is technically very demanding as it requires mass-spectrometry based methods. Thus, currently it is not suitable for clinical use but may be in future.

6.4 Treatment of Invasive Aspergillosis

Choosing the proper antifungal agent for treatment of IA may be complex. Several factors have to be considered and different classes of antifungal agents with mold activity are available. Factors that need to be integrated in the decision making process when it comes to IA treatment include the underlying disease, potential side effects of antifungals, drug-drug interactions, concomitant medication that may be contraindicate the use of distinct antifungals (e.g. chemotherapy), previous fungal infections, previous treatment with antifungals, potential need for therapeutic drug monitoring (TDM) and of course, costs of antifungals. Underlying antifungal prophylaxis, a common approach for prevention of IFIs in high risk patients^(94, 144), has also to be considered when choosing antifungal agents for therapy. In a patient with fluconazole prophylaxis developing breakthrough IFI for example, it is highly suspected that this IFI is either caused by fluconazole resistant *Candida* or by molds. Thus, empirical therapy in this case has to cover both possible scenarios. Switch to another class of antifungals in a patient already receiving mold active antifungals is recommended if possible⁽¹⁹⁴⁾.

Currently available antifungals that can be used for treatment of IA are polyenes (amphotericin B formulations), azoles (voriconazole, posaconazole and isavuconazole) and echinocandines (anidulafungin, caspofungin and micafungin) which are going to be discussed here. Currently available guidelines for treatment of IA are the 2016 “Recommendations for Diagnosis and Treatment of Aspergillosis” published by the IDSA⁽⁹⁴⁾ and the European Conference on Infections in Leukaemia-6 (ECIL) guidelines for treatment of invasive candidiasis, aspergillosis and mucormycoses in leukemia and HSCT patients⁽¹⁹⁵⁾. The long awaited treatment guideline from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) is still pending but expected to be published this year.

6.4.1 Lipid Amphotericin B Formulation

Conventional amphotericin B (AmB) is associated with high frequency of nephrotoxicity and other potentially severe adverse events. With the introduction of lipid based formulations, liposomal amphotericin B (lipAmB), these side effects have significantly lessened⁽¹⁹⁶⁾. The primary mechanism of AmB action is that AmB binds to ergosterol and

other sterols leading to formation of pores in the fungal cell wall with leakage of cellular components and consequent cell death. Recent findings, however, suggest an (additional) mechanism. AmB forms extramembranous aggregates that are able to extract ergosterol from the cell wall and destroy the cell wall integrity⁽¹⁹⁷⁾. As all AmB formulations demonstrate poor oral uptake, they have to be administered either intravenously or per inhalation. The approved lipAmB dosage for IA treatment is 3-5 mg/kg/day^(94, 198). In the AmBiLoad Trial, a double-blind dose finding trial, 3 mg/kg/day of lipAmB were compared to a high-dose therapy with 10 mg/kg/day of lipAmB in 201 highly immunocompromised patients with proven or probable mold infections⁽¹⁹⁹⁾. The primary outcome, complete or partial response of the infection, was reached by 50% in the low-dose group and 47% in the high-dose group ($p>0.05$). There was also no difference in 12-week survival rate, but significantly higher rates of nephrotoxicity and hypokalemia in the high-dose group. Based on these observations, a higher dose of lipAmB $>3-5$ mg/kg/day is generally not recommended for treatment of IA. High-dose lipAmB (5-10 mg/kg/day) is considered for treatment of mucormycoses that may be a cause of breakthrough IFI in patients receiving voriconazole prophylaxis or therapy^(195, 200).

6.4.2 Echinocandins

Currently, three echinocandins are available: anidulafungin, caspofungin and micafungin. Even though echinocandins have their major clinical role in treatment of *Candida* infections they all exhibit *Aspergillus* activity⁽²⁰¹⁻²⁰³⁾. As with lipAmB, echinocandins have to be administered intravenously. They act via inhibition of glucan synthase, which is needed for production of 1,3- β -D-glucans which are essential for cell wall integrity^(94, 204). Echinocandins do not act fungicidal but highly alter the growth of the fungal cell wall. Side effects of echinocandins are uncommon and they are usually well tolerated. Among all available antifungal groups, echinocandins have the lowest potential of hepatotoxicity which is important for treatment of IA in patients with underlying liver diseases^(205, 206). Dose adjustment is recommended for caspofungin in patients with liver cirrhosis grade B or C according to the Child-Pugh score and micafungin is not recommended in patients with Child-Pugh C cirrhosis. Anidulafungin does not require dose adjustment in case of liver cirrhosis⁽²⁰⁷⁾. In a meta-analysis of hepatotoxicity of antifungals⁽²⁰⁶⁾, echinocandins caused treatment discontinuation due to drug induced liver injury in 4.8% (95%

confidence interval 3.0 – 6.5), which was significantly less compared to lipAmB and voriconazole (12.7% and 14.7%, respectively). Overall the pooled estimated risk for drug induced liver injury and for consequent treatment discontinuation was 7% for caspofungin, 3% for micafungin and 2% for anidulafungin ⁽²⁰⁶⁾. However, echinocandins are not recommended as first line therapy for IA, as there is currently no randomized trial investigating safety and efficacy for IA therapy. Evidence that, even in case of lacking comparative trials, echinocandins are an alternative treatment option for IA therapy in case recommended first line therapies are contraindicated, however, is growing ⁽²⁰⁸⁾.

Currently there is an ongoing phase 3 study, investigating the efficacy and safety of an oral glucan synthesis inhibitor (SCY-078) that may be used in clinical routine in future (ClinicalTrials.gov Identifier: NCT03059992).

6.4.3 Azoles

Azoles are broad spectrum antifungal agents and act via inhibition of the cytochrome P450-dependent enzyme lanosterol 14- α -demethylase, leading to a lack of conversion from lanosterol to ergosterol ⁽²⁰⁹⁾. A lack of ergosterol, as already mentioned, leads to loss of cell wall integrity and consecutive death of the fungal cell. To *Candida* species, triazoles are usually acting fungistatic, whereas voriconazole has been shown to act fungicidal against *Aspergillus* species ⁽²¹⁰⁾. Voriconazole, posaconazole, isavuconazole and itraconazole have *Aspergillus* activity, whilst fluconazole does not have any activity against *Aspergillus* and is used for treatment of *Candida* infections.

All azoles are available in intravenous solution and in oral administration form (oral suspension or tablet). Therefore, azoles are currently the only option for oral treatment or prophylaxis of IA.

6.4.3.1 Voriconazole

Voriconazole is considered the first line therapy for IA for most patients ^(94, 195). Voriconazole is extensively metabolized in the liver by primarily CYP2C19 but also by CYP3A4 ⁽²¹¹⁻²¹³⁾. It is not only substrate but also inhibitor of these cytochromes. Only 5% of voriconazole are eliminated via the kidneys. This extensive hepatic metabolization leads to significant plasma level variations in case of polymorphisms in CYP2C19 ⁽²¹⁴⁾. Several other factors may also influence voriconazole pharmacokinetics, so measurement of serum

levels is recommended to guarantee adequate drug levels for treatment and to reduce potential toxicity⁽⁹⁴⁾. The most common adverse event in voriconazole therapy is transient and reversible visual disturbances indicating a high serum concentration, reported in up to 30% of patient on voriconazole⁽²¹⁵⁻²¹⁸⁾. Other studies, however, report as primary side effect liver abnormalities^(219, 220). Mild hepatotoxicity is a common side effect of voriconazole treatment, whereas severe hepatotoxicity is uncommon⁽²²¹⁻²²³⁾.

Voriconazole is recommended as first line therapy for IA based on a comparative study of voriconazole versus conventional AmB⁽²²⁰⁾. This study randomized 277 patients with mixed underlying diseases in either voriconazole or AmB group. Successful outcome was significantly better in the voriconazole group (52% versus 31%). Similar findings were reported in open label studies^(224, 225). Based on these data the FDA approved voriconazole as first line therapy for IA treatment.

6.4.3.2 Posaconazole

Posaconazole is another broad-spectrum azole. It is available as oral suspension, as gastro-resistant tablet and as intravenous solution and currently approved for antifungal prophylaxis in patients with prolonged neutropenia and in patients with acute graft-versus-host diseases (GVHDs) after HSCT⁽²²⁶⁻²²⁸⁾. TDM is necessary when using posaconazole as prophylaxis, as plasma levels may vary significantly from patient to patient and may only be achieved at approximately one week after initiation of therapy. Plasma levels on days three to five, however, may predict satisfactory posaconazole plasma levels⁽²²⁹⁾. Posaconazole is not recommended for first-line therapy of IA due to lack of comparative trials with voriconazole. Such study is currently ongoing (ClinicalTrials.gov Identifier: NCT01782131) and results are expected for 2019.

6.4.3.3 Isavuconazole

Last but not least, isavuconazole the newest member in the family of azoles. It has a long half-life of 5-days that permits once-daily dosing after a loading dose give for two days. Compared to voriconazole, isavuconazole brings the advantage of a wider antifungal spectrum, a linear pharmacokinetic (may waiving the need of TDM) and fewer CYP enzyme mediated drug-drug interactions⁽²³⁰⁾. Besides treatment for IA, isavuconazole is also licensed as a second-line therapy for mucormycoses⁽¹⁰⁰⁾. In the SECURE trial, isavuconazole was compared to voriconazole for first-line IA therapy and was shown to be non-inferior⁽¹⁰⁰⁾. Less eye disorders, hepatobiliary disorders and skin and soft tissue

disorders were reported in the isavuconazole group. More than that, less drug-drug interactions (42% versus 60%) and fewer treatment discontinuations due to adverse events (14% versus 23%) were reported in the isavuconazole group. Thus, isavuconazole is recommended as first line therapy for IA in leukemia patients and HSCT recipients ⁽¹⁹⁵⁾. The IDSA recommends isavuconazole as primary alternative to voriconazole ⁽⁹⁴⁾ which could be of interest in liver cirrhosis patients considering the better hepatobiliary side effect profile of isavuconazole.

6.4.4 Combination Therapy

Combination therapy of different antifungal agents is not recommended as a first-line therapy approach due to lack of evidence but may be considered in selected patients ⁽⁹⁴⁾. Most combination approaches were performed by using an azole (primarily voriconazole) and an echinocandin. The majority of studies were retrospective studies and only two had a prospective, randomized design. The first one was an open label pilot study comparing lipAmB low-dose (3 mg/kg/day) plus caspofungin at standard dose versus high-dose lipAmB (10 mg/kg/day) monotherapy ⁽²³¹⁾. Survival was similar in both groups without significant differences but response to therapy was better in the combination group (67% versus 27%; $p=0.028$). Recently, a prospective, placebo controlled study was published comparing voriconazole monotherapy to voriconazole and anidulafungin combination therapy ⁽²³²⁾. There was a trend towards higher 6-weeks survival rate in the combination group (19.3% versus 27.5%; $p=0.087$). In post hoc analyses a significantly better survival was noted for the combination therapy in the subgroup of patients who were diagnosed as having probable IA based on radiological pathologies and a positive GM result. As this study could not clearly prove a benefit for combination therapy but showed that this may be a treatment option for selected patients the IDSA recommended to consider a combination of voriconazole and an echinocandin in case of severe disease and severe underlying illness (mainly severe neutropenia).

6.5 Invasive Aspergillosis and Liver Cirrhosis

6.5.1 Why are Patients with Liver Cirrhosis at Risk for Invasive Aspergillosis?

Liver cirrhosis is a well-known cause for impaired immune dysfunction ⁽²³³⁾. The importance of the liver as essential part of the immune system is based on two features of the liver. First, the liver is a kind of sentinel station for immune surveillance. Kupffer cells, a group of macrophages based in the liver, sinusoidal endothelial cells and dendritic cells are constantly observing the blood stream for microbial pathogens. In case Kupffer or sinusoidal endothelial cells may bind a recognized pathogen, they are presenting antigens via major histocompatibility complex (MHC) class I and II molecules and trigger further immune response ^(234, 235). Second, the liver is one of the most important producers of soluble PRRs, with C-reactive protein (CRP) as the most prominent representor ⁽²³⁶⁾. Besides soluble immune system components, also membrane-bound PRRs are expressed that are supposed to bind recognized PAMPs. Risk for infections in cirrhotic patients result from impairments of several of these complex immunes mechanisms. Structural derangement within fibrosis and cirrhosis and consecutive porto-systemic shunt, loss of function of the reticulo-endothelial system due to sinusoidal fibrosis as well as impaired function of Kupffer cells are observed within liver cirrhosis and contribute to high infection risk ^(237, 238). In addition, the production of soluble immune system components and PRRs, which are essential of recognition of bacterial and fungal structures, are impaired. Of special interest for susceptibility to fungal infections is the impaired function of neutrophils that comes along with liver cirrhosis. Neutrophils are often reduced in number due to pooling and sequestration in the spleen and have also reduced phagocytic activity ⁽²³⁹⁻²⁴²⁾. Impaired chemotaxis and migration in neutrophils additionally contribute to their impaired activity ^(243, 244). Several other factors like impaired function of monocytes, B lymphocytes, T lymphocytes and natural killer cells are observed in liver cirrhosis of which all may contribute to a higher vulnerability to infections, including fungal infections.

6.5.2 Why is the Prevalence of Invasive Aspergillosis Unknown in Patients with Liver Cirrhosis?

Clinical signs and symptoms of IA are generally non-specific, often with an insidious onset but a fatal outcome⁽³²⁾. In addition, several characteristics of cirrhotic patients may make the diagnosis of infections in general and of IA in particular difficult: blunted elevation of body temperature, baseline elevated heart rate because of the hyperdynamic circulatory syndrome and/or baseline hyperventilation due to hepatic encephalopathy and elevated inflammation biomarkers without underlying infection⁽²⁴⁵⁻²⁴⁸⁾. Definite diagnosis of IA is established only post mortem in more than 50% of cases^(32, 36, 249). However, the rate of autopsies has declined over the past decades, although rates may vary from institution to institution^(249, 250). Even if autopsies are performed poor sampling, failure to use special stains for *Aspergillus* or lack of microbiological workup may lead to missed cases of IA. Furthermore, cultures for *Aspergillus* from respiratory tract specimen in vivo have a low diagnostic sensitivity and do not prove the presence of invasive infection⁽²⁵¹⁾. In addition, screening for IA is uncommon among patients with decompensated liver diseases. Taken together, these factors may explain why IA may be underdiagnosed in this patient group.

Thus, the aim of this study was to explore the prevalence of IA in patients with underlying liver cirrhosis. In addition, the performance of serum GM screening, a common approach for early IA diagnosis in immunocompromised patients, for IA diagnosis in these patients was evaluated.

7 Materials and Methods

7.1 Study Design

This study was an exploratory, prospective, mono-centric study conducted at the University Hospital of Graz, Austria. From August 2013 to December 2015, 154 consecutive patients were included in this study. Results from this study were published recently ⁽²⁵²⁾.

7.2 Patients

Patients were recruited at the University Hospital of Graz, Austria by daily screening (from Monday to Friday and retrospectively on Monday for the preceding weekend) of patient protocols at the Division of Gastroenterology and Hepatology, Intensive Care Unit (ICU) and Emergency Department (all Department of Internal Medicine) and by twice weekly chart reviewing at the Division of Transplant Surgery, Department of Surgery. All adult patients ≥ 18 years of age with underlying liver cirrhosis, who were admitted to our hospital were considered eligible for study inclusion and underwent screening for inclusion criteria. After patients were included once in our study, they were not considered for a second time inclusion in case of re-admission to the hospital ⁽²⁵²⁾.

Inclusion criteria:

- I.) Age ≥ 18 years
- II.) Underlying decompensated liver cirrhosis regardless of reason for admission and clinical symptoms or compensated liver cirrhosis with respiratory symptoms (cough, dyspnea, pleuritic pain, hemoptysis) and/or fever
- III.) Written informed consent

Compensated and decompensated liver cirrhosis was defined by using a previously published classification system ⁽²⁵³⁾. Four stages of cirrhosis have been proposed based on features of portal hypertension ⁽²⁵³⁾.

- Stage I is defined by the absence of ascites or varices.
- Stage II is characterized by the absence of ascites and the presence of varices that have never bled. Stages I and II represent compensated cirrhosis.
- Stage III is defined by the presence of ascites with or without varices that have never bled.
- Stage IV is characterized by the presence of variceal bleeding in patients with or without ascites. Stages III and IV represent decompensated cirrhosis ⁽²⁵⁴⁾.

If patients were not able to give informed consent at the time of admission and study inclusion (e.g. due to hepatic encephalopathy or poor clinical condition), they were informed and asked for their consent as soon as their condition improved and could withdraw from the study at this point according to the approval of our ethics committee and were excluded at that time point from this study and from further analyses.

7.3 Sampling and Diagnostic Management

The first serum sample for galactomannan-antigen-testing (GM) was collected from patients within 24h after study inclusion and twice weekly thereafter until patients were discharged or died ⁽²⁵²⁾. Serum GM screening was reduced from twice weekly to once weekly from week five on, in case patients were hospitalized for four weeks or longer without signs or symptoms of IA and constantly negative serum GM results within the first four weeks. A positive serum GM result triggered performance of HRCT of the chest to rule out or confirm pulmonary IA. CT scans were also performed in case of clinical suspicion for presence of IA or alternative pulmonary infections at the discretion of the treating physician. In case radiographic signs suggestive of pulmonary IA were observed on imaging studies [i.e. small nodules (<1 cm), consolidations, large nodules (≥ 1 cm), peribronchial infiltrates, ground-glass infiltrates, halo sign, air crescent sign ^(117, 255)], bronchoscopy with BALF sampling, GM determination in BALF and BALF culture were performed. Consistent positive serum GM and lack of radiological signs on chest HRCT scan triggered consecutive CT scans of the paranasal sinuses, the cranium and abdomen to check for alternative sites of infection. GM concentrations were determined by using the Platelia *Aspergillus* EIA (Bio-Rad, Vienna, Austria) test kits according to the manufacturer's instructions at the Institute of Hygiene, Microbiology and Environmental

Medicine, Medical University of Graz, Austria. GM results with an ODI ≥ 0.5 were considered positive in case of serum samples and ≥ 1 in case of testing BALF samples ⁽²⁵²⁾. For each run of GM testing, a positive, a negative and two cut-off controls were tested to validate the test results and calculate ODI for samples. All positive samples were tested twice. Serum and BALF-GM testing was performed according to the manufactures instructions:

All samples and reagents were first brought to room temperature. In a first step, 300 μL of serum or BALF samples, positive controls, negative controls and cut-off controls were transferred to individual polypropylene tubes. In the second step, 100 μL of provided Sample Treatment Solution (EDTA acid solution) was added to each tube, vortexed and heated in a water bath for three minutes at 100°C. After heating, tubes were centrifuged at 10,000 x g for 10 minutes. GM determination was then performed by using the supernatant. For this purpose, 50 μL of the Conjugate Solution (anti-galactomannan monoclonal antibody/peroxidase labeled) were transferred to each well of the 90-well plate. After that, 50 μL of the serum or BALF supernatant were added to these wells, covered with a plate sealer and incubated for 90 minutes at 37°C. After incubation the plate was washed 5 times with 800 μL Washing Solution and 200 μL of Chromogen TMB Solution [3,3',5,5'-tetramethylbenzidine (<0.1%); H₂O₂ (<1.0%)] was added. The microplate was again incubated at + 18-20°C in a dark room to avoid light exposure for 30 minutes. Following this final incubation step, 100 μL Stopping Solution [1 N sulphuric acid solution (H₂SO₄)] was added to each well. Finally, the ODI was read out of each well at 450 nm. A valid result needs a valid positive and negative control. Positive controls have to yield an index > 1.5 and negative controls <0.4. The ODI for the tested samples is finally calculated by using following formula:

$$\text{ODI} = \frac{\text{Optical density sample}}{\text{Mean cut off control optical density}}$$

Mean cut-off control is calculated by adding the optical densities of the two wells containing Cut-Off Control Serum and divide the result by 2.

Serum GM performance was calculated for all included patients, and for two subgroups: I.) patients presenting with respiratory symptoms; II.) patients with clinical suspicion for IA. I.) All patients presenting with respiratory symptoms (new cough, dyspnea, and hemoptysis or pleuritic chest pain) with or without clinical suspicion of presence of IA and irrespective of whether chest CT scan was performed, were included in the first subgroup analysis for serum GM performance. II.) Clinical suspicion for IA was based on the clinical suspicion of the treating physician for presence of pulmonary infection and consecutive performance of a chest CT scan. Decision whether or not to perform CT scan in this subgroup was, therefore, not triggered by screening for IA within the study protocol (252)

7.4 Diagnostic Criteria

IA was classified according to a slightly modified version of the European Organization of Research and Treatment of Cancer/Mycoses Study Group revised definitions of 2008 that includes modified host criteria as displayed in table 3 (252). The list of host factors for probable and possible IA was extended by liver cirrhosis. Modifications were necessary as host factors in the original definitions were defined for patients with underlying hematological malignancies and other severely immunocompromised patients (33, 252, 256). However, they have not been evaluated in patients at risk of IA without underlying hemato-oncological malignancies. Similar approaches have been chosen in previous studies evaluating prevalence of IA in non-neutropenic patients (42, 91, 192).

Table 3. Diagnostic criteria for invasive aspergillosis used in this study (252)

Proven IA

Histopathologic, cytopathologic or direct microscopic examination of tissue invasion by septated, acutely branching filamentous fungi and/or by a positive culture for *Aspergillus* in normally sterile specimen

Probable IA

Host risk factor: liver cirrhosis

AND

Clinical features: abnormal radiological imaging compatible with pulmonary or extrapulmonary (in

first line cerebral or sinonasal) infection with molds

AND

Mycological criteria: direct test (cytology, direct microscopy or cultures) indicating the presence of *Aspergillus* species or galactomannan antigen detected in serum or BALF

Possible IA

Presence of a host factor and classical radiological features (dense, well-circumscribed lesion(s) with or without halo sign, air-crescent sign and cavity), but absence of mycological criteria

7.5 Statistics

For statistical analysis, SPSS 23 (SPSS Inc., Chicago, IL) was used. Continuous variables are displayed as means plus 95% confidence interval (95% CI) or median plus 25 and 75 quartiles (IQR) as appropriate. 95% CI for IA prevalence was calculated by using the Wilson score method ⁽²⁵⁷⁾. Categorical variables are displayed as absolute number plus percentage. For serum GM performance sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the overall cohort and for patients classified as having clinical suspicion for IA were calculated. 90 days survival rate was calculated by Kaplan-Meier survival curve. Survival curves were compared by using the Log-rank test. This study was conducted in accordance with the Declaration of Helsinki, 1996, Good Clinical Practice, and applicable local regulatory requirements and law. The study protocol was approved by the local ethics committee, Medical University Graz, Austria (EC-number 25-326 ex 12/13).

8 Results

154 patients were prospectively enrolled in this study. 150 patients (97.4%) were included in the analysis. Four patients (2.6%) had to be excluded retrospectively due to following reasons: 1.) liver cirrhosis was mentioned as underlying disease in the emergency department but was retrospectively rejected after diagnostic work up (n=2), 2.) patient death before the first serum GM screening sample was drawn (n=1) and 3.) patient who was included in the study within an episode of hepatic encephalopathy and not able to give informed consent at the time of inclusion refused to give informed consent after clinical improvement (n=1) ⁽²⁵²⁾.

Demographic data and characteristics of study participants are displayed in table 4.

Table 4. Demographic data and baseline characteristics of the study population classified according to the modified EORTC/MSG 2008 criteria . Reproduced from ⁽²⁵²⁾ with permission of Oxford University Press

Patient Characteristics	Overall study cohort	Probable IA cases	Possible or no IA
No. of patients	150 (100)	2/150 (1.3)	148/150 (98.7)
Sex			
Male	121 (80.7)	2/121 (1.7)	119/121 (98.3)
Female	29 (19.3)	0/29	29/29 (100)
Age, years	58 (50-64)	55 (54-55)	58 (50-65)
Baseline characteristics			
Decompensated cirrhosis	140 (93.3)	0/140	140/140 (100)
Compensated cirrhosis	10 (6.7)	2/10 (20)	8/10 (80)
Fever (>38.5°C)	7 (4.7)	0/7	7/7 (100)
Respiratory Symptoms [#]	39 (26)	1/39 (2.6)	38/39 (97.4)
Dyspnea	23 (15.3)	0/23	23/23 (100)
Cough	20 (13.3)	0/20	20/20 (100)
Hemoptysis	1 (0.7)	1/1 (100)	0/1
White blood cells, 10 ³ /μL	6.1 (4.5-8.4)	5.5 (2.1-8.9)	6.1 (4.5-8.4)

C-reactive protein level, mg/L	15.9 (6.5-35.6)	37.4 (20.5-54.3)	15.6 (6.4-35.5)
Etiology of cirrhosis			
Alcoholic liver disease	97 (64.7)	1/97 (1)	96/97 (99)
Viral hepatitis B or C	20 (13.3)	0/20	20/20 (100)
Mixed conditions [§]	6 (4)	0/6	6/6 (100)
NAFLD	6 (4)	0/6	6/6 (100)
Primary/secondary biliary cirrhosis	5 (3.3)	1/5 (20)	4/5 (80)
Others or unknown	16 (10.7)	0/16	16/16 (100)
Child-Pugh A	11 (7.3)	1/11 (9.1)	10/11 (90.9)
Child-Pugh B	70 (46.6)	1/70 (1.4)	69/70 (98.6)
Child-Pugh C	69 (46)	0/69	69/69 (100)
MELD score, points	16 (13-22)	15.5 (14-17)	16 (13-22)
Underlying risk factors for IA			
Neutrophils <500/ μ L	0	0	0
COPD	20 (13.3)	0/20	20/20 (100)
Asthma	1 (0.7)	0/1	1/1 (100)
Diabetes mellitus	30 (20)	1/30 (3.3)	29/30 (96.7)
Active malignancies	20 (13.3)	0/20	20/20 (100)
Immunosuppressive therapy			
Glucocorticoid treatment	8 (5.3)	0/8	8/8 (100)
High dose glucocorticoid treatment*	3 (2)	0/3	3/3 (100)
Azathioprine	1 (0.7)	0/1	1/1 (0.7)
GM tests per patient [§]	3 (3-4)	10 (7-13)	3 (2-4)
Length of stay, days	11.5 (7-21)	35 (7-63)	11.5 (7-20.8)

ICU admission	34 (26.7)	0/34	34/34 (100)
Length of ICU stay, days	3 (2-6)	-	3 (2-6)
90-days mortality	25 (16.7)	0/25	25/25 (100)

Data are given as median (interquartile range) or absolute counts (%)

Five patients had cough and dyspnea.

§Implicating chronic viral hepatitis and a history of alcohol abuse

* = mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for ≥ 3 weeks ⁽³³⁾

[§]Serum GM samples tested per patient

Abbreviations: EORTC/MSG = EORTC/MSG = European Organization for Research and Treatment of Cancer/Mycosis Study Group;

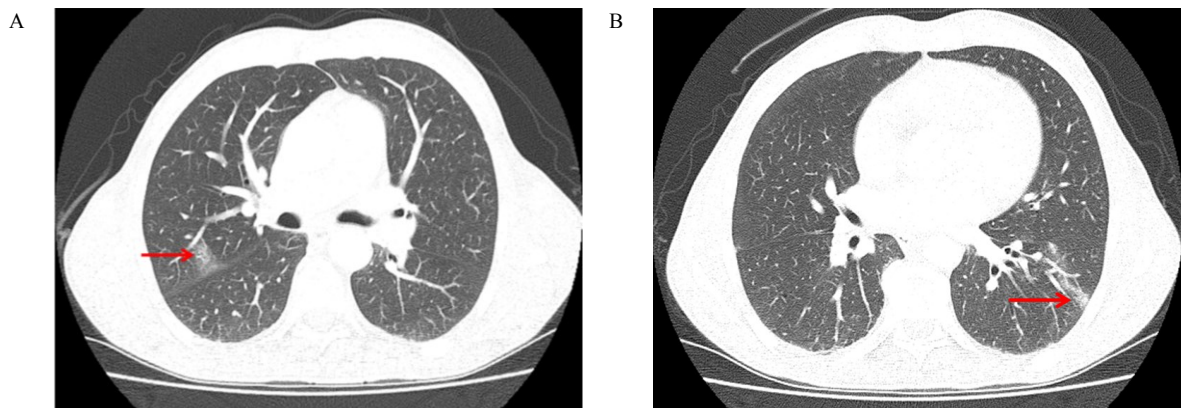
IA = invasive aspergillosis; NAFLD = nonalcoholic fatty liver disease; MELD = model for end stage liver disease; COPD = chronic obstructive pulmonary disease; GM = galactomannan; ICU = intensive care unit

8.1 Patients with Probable and Possible Invasive Aspergillosis

Two patients were diagnosed with probable IA (1.3%; 95% CI 0.37 – 4.7), one patient with possible IA (0.7%; 95% CI 0.1 – 3.7) and no evidence of IA was found in the remaining 147 patients (98%; 95% CI 94.3 – 99.3). Both patients with probable IA had compensated liver cirrhosis in Child-Pugh stage A. Patient 1 had underlying secondary biliary cirrhosis (Child-Pugh score of 9; model for end-stage liver disease score of 17) due to IgG4-related sclerosing cholangitis and well-controlled insulin depended type 1 diabetes mellitus [glycated hemoglobin (A1C) 24 mmol/mol]. Few weeks prior to admission and study inclusion, the patient received percutaneous transhepatic cholangiography and placement of a percutaneous biliary catheter for drainage purpose due to his underlying sclerosing cholangitis. *Candida albicans* was cultured in a single culture from the bile and the patient was set on fluconazole 200 mg twice daily from this time point on. Serum BDG was not performed as it was not available routinely at this time at our institution (summer 2013). He was than readmitted for pre-liver transplant evaluation one week prior to study inclusion. At readmission CRP levels were elevated (71 mg/L; upper limit of norm = 5 mg/L) without clinical signs of infection and levofloxacin was added to fluconazole. Baseline neutrophils were $1.3 \times 10^3/\mu\text{L}$. Positive serum GM (0.6), performed after study inclusion, triggered chest HRCT, revealing multiple, bilobular areas of consolidation

(figure 9). Based on positive serum GM, findings on HRCT scan and underlying liver cirrhosis the patient was diagnosed with probable IA and therapy was switched from fluconazole to voriconazole. Bronchoscopy was routinely scheduled and performed nine days after initiation of voriconazole therapy. BALF-GM determination revealed a negative result (0.38 ODI). All consecutively performed serum GM levels were negative. Voriconazole therapy was continued for a total of 54 days before discontinuation. The patient was doing well after discontinuation of voriconazole and received a liver transplant six months after study inclusion.

Patient 2 had underlying alcoholic liver cirrhosis and ongoing alcohol abuse (Child-Pugh score of 5, model for end-stage liver disease score of 14). The patient was admitted to our hospital due to sudden onset hemoptysis since one day. He had an ongoing history of smoking (approximately 30 pack years) but no history of COPD and no ongoing inhaled therapy. All serum GM test results remained negative. Baseline neutrophils were $5.8 \times 10^3/\mu\text{L}$. HRCT scan of the chest was performed because of hemoptysis, showing single ground-glass opacity with 1.8 cm in diameter in the left upper lobe (figure 10). Bronchoscopy yielded tracheobronchitis and BALF-GM was 3.44. In further diagnostic work up pulmonary malignancy could be ruled out. Voriconazole was initiated and administered for 14 days. In the follow-up CT scan, performed three days after discontinuation of voriconazole therapy, the previously described opacity had resolved (figure 10).



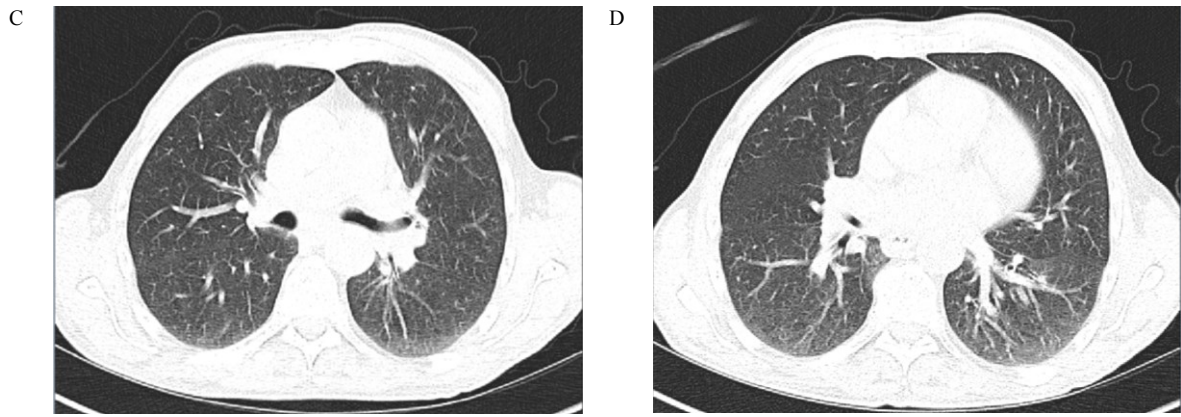


Figure 9. Chest computed tomography scans of patient 1 with probable invasive aspergillosis. Figures A and B demonstrating bilobular areas of consolidation (red arrows). After 54 days of voriconazole therapy these consolidations have resolved (C and D).

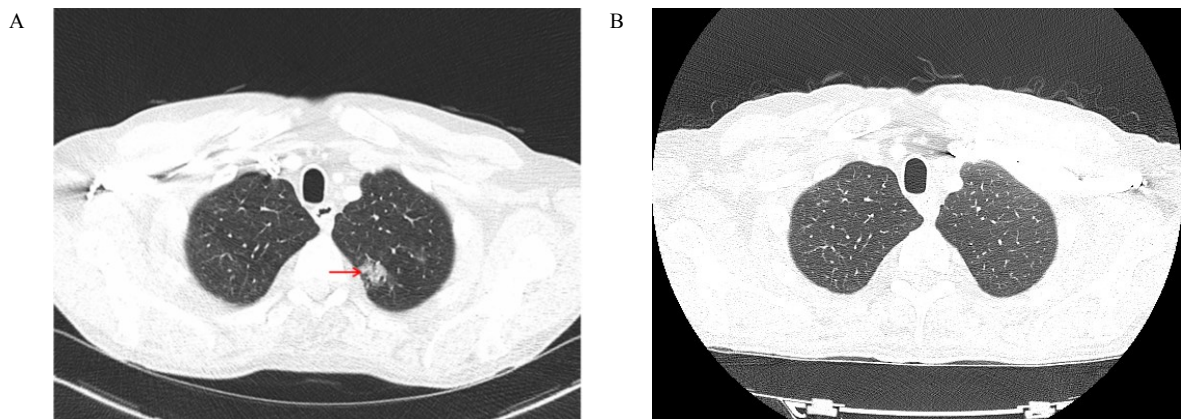


Figure 10. Chest computed tomography scans of patient 2 with probable invasive aspergillosis. Figure A shows ground-glass opacity in the left upper lobe without a halo sign (red arrow). After 14 days of voriconazole therapy the lesion resolved and the patient was free of complains.

Patient 1 and 2 received oral voriconazole therapy for approximately six and two weeks, respectively, with a loading dose of 400 mg twice daily on day 1 followed by a maintenance dose of 200 mg twice daily. As suggested for voriconazole, therapeutic drug monitoring was performed regularly in both patients. In patient 1 six out of nine (66.7%) samples were in the range of 1-5.5 mg/L, two were below 1 mg/L and one was higher than 5.5 mg/L. In patient 2 voriconazole levels were checked three times within two weeks of therapy, and were within the range of 1-5.5 mg/L at all times. Voriconazole therapy was well tolerated in both patients, aside from a temporary blurred vision in patient 1.

Bilirubin, aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) levels before, during and after voriconazole therapy for both patients are displayed in figure 11. Both patients improved clinically after voriconazole was initiated and survived.

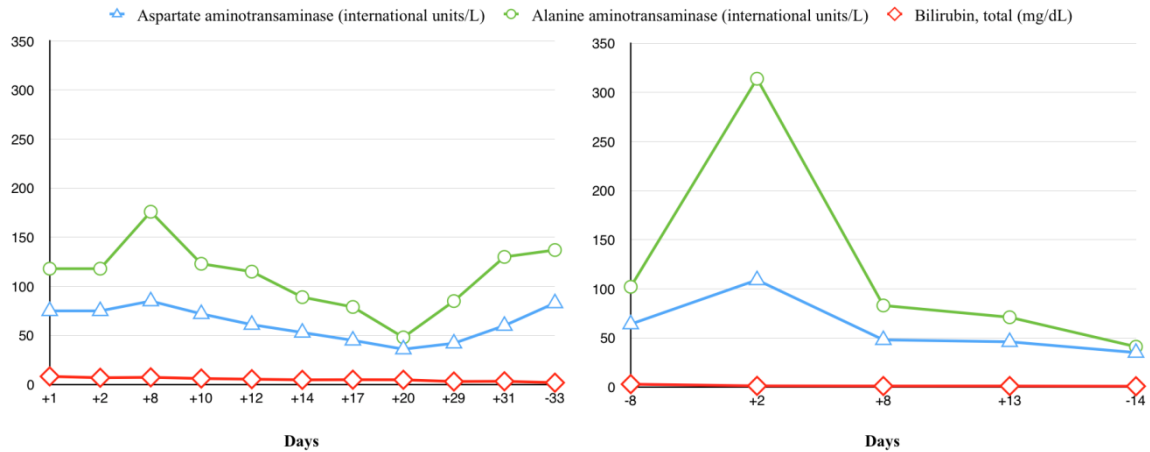


Figure 11. Liver function tests for both patients with probable invasive aspergillosis and voriconazole therapy. Both patients showed an increase in alanine aminotransaminase and aspartate aminotransaminase. Discontinuation of voriconazole was not necessary and levels of aminotransaminases declined to baseline levels despite ongoing therapy. “-” days are indicating days before initiation and after discontinuation of voriconazole therapy. “+” days are indicating number of days since initiation of voriconazole therapy.

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One patient was diagnosed with possible IA based on a positive host factor and findings on chest CT scan. The patient suffered from IgG4-related sclerosing cholangitis and iridozyclitis. Due to the presence of iridozyclitis the patient had an ongoing systemic prednisone therapy (0.11 mg/kg/d) for more than a year at the time of presentation. The patient presented to the emergency department because of headache since approximately 2 weeks. Besides an elevated serum CRP level (212 mg/L), initial laboratory work up was inconspicuous. Lumbar puncture was performed due to the headache and systemic infection signs but turned out unsuspecting. Due to elevated CRP chest x-ray was performed to rule out pneumonia. Chest x-ray revealed a macronodule in the right upper lung with 4.2 x 1.9 cm in diameter. Consequently, chest HRCT scan was performed and confirmed presence of two macronodules without a halo sign in the right upper lung. Due to unclear etiology of lung nodules the patient was put on intravenous voriconazole with 6 mg/kg on day 1 and 4 mg/kg from day 2 on and piperacillin/tazobactam. For further diagnostic work-up, bronchoscopy was performed two days after admission and after initiation of empirical voriconazole therapy. BALF-GM as well as serum GM and BDG

yielded, however, negative results. Thus, voriconazole was discontinued after three days. CT guided puncture of the nodules showed no evidence for fungal infection but presence of inflammatory IgG4-related pseudotumor. The patient improved clinically with piperacillin/tazobactam and without antifungal therapy.

8.2 Serum Galactomannan Performance

During the study 505 serum samples (median 3 per patient; range 1 - 13) and five BALF samples obtained from five patients were tested for presence of GM. Positive serum GM results were observed in six patients [five without evidence for IA and one with probable IA (patient 1)].

Serum GM sensitivity for probable versus no IA for the overall cohort was 0.5 (95% CI 0.09 – 0.91), specificity was 0.97 (95% CI 0.92 – 0.99), NPV 0.99 (95% CI 0.96 – 0.99) and PPV 0.17 (95% CI 0.01 – 0.64). 39/150 (26%) patients had underlying respiratory symptoms (=subgroup I). One patient within this group had probable IA. Serum GM performance for probable versus no IA in patients with respiratory symptoms was as following: sensitivity 0, specificity 0.92 (95% CI 0.78 – 0.98), NPV 0.97 (95% CI 0.84 – 1.00) and PPV 0. 13/150 (8.7%) patients were characterized as having clinical suspicion for IA (=subgroup II). Two patients with clinical suspicion of IA had probable IA. Serum GM performance for probable versus no IA in patients with clinical suspicion for IA was as following: sensitivity 0.50 (95% CI 0.03 – 0.99), specificity 0.90 (95% CI 0.54 – 0.99), NPV 0.90 (95% CI 0.54 – 0.99) and PPV 0.50 (95% CI 0.03 – 0.98). Serum GM performance calculation for the overall study cohort, for patients with respiratory symptoms and for patients with clinical suspicion for IA are summarized in table 5.

False positive serum GM results were observed in 5/150 patients (3.3%) without evidence for IA. None of these patients had received piperacillin/tazobactam. Only one positive test result (1.36 and 0.59, respectively) with consecutive negative controls was observed in two patients. In another two patients two consecutive serum GM results turned out positive (0.56 and 0.54 and 0.53 and 1.2, respectively) followed by negative controls without initiation of antifungal therapy. One patient, interestingly, had ten consecutive positive serum GM results (range 0.71 – 4.58) without a single negative GM test result within the whole follow up period. In this particular patient all other testings including serum BDG, serum *Aspergillus* specific lateral-flow device test as well as whole blood *Aspergillus*

specific and panfungal real-time polymerase chain reaction turned out negative. CT scans of paranasal sinuses, neurocranium, chest and abdomen were inconspicuous and showed no evidence of fungal infection, thus antifungal therapy was withheld. Despite the lack of antifungal therapy the patient did not develop an invasive fungal infection, received liver transplantation and survived. Reasons for constantly elevated serum GM levels remained unclear.

Table 5. Performance of serum galactomannan testing in various subgroups. Reproduced from ⁽²⁵²⁾ with permission of Oxford University Press

	Prevalence of Invasive Aspergillosis	Sensitivity	Specificity	Negative Predictive Value	Positive Predictive Value
Overall study cohort (n=150)	2/150 (1.3%)	0.5 (0.09 – 0.91)	0.97 (0.92 – 0.99)	0.99 (0.96 – 0.99)	0.17 (0.01 – 0.64)
Patients with respiratory symptoms (n=39)	1/39 (2.6%)	0	0.92 (0.78 – 0.98)	0.97 (0.84 – 1)	0
Clinical suspicion for IA (n=13)	2/13 (15.4%)	0.5 (0.03 – 0.99)	0.9 (0.54 – 0.99)	0.9 (0.54 – 0.99)	0.5 (0.03 – 0.98)

8.3 Mortality

The overall 90-day mortality rate in this study was 16.7% (25/150). Median time from study inclusion to death was 42 days (IQR 21-66). One patient died within 24h of admission and study inclusion. Both patients with probable IA and the one patient with possible IA survived. Kaplan-Meier cumulative survival curves for patients at various Child-Pugh stages are displayed in figure 12.

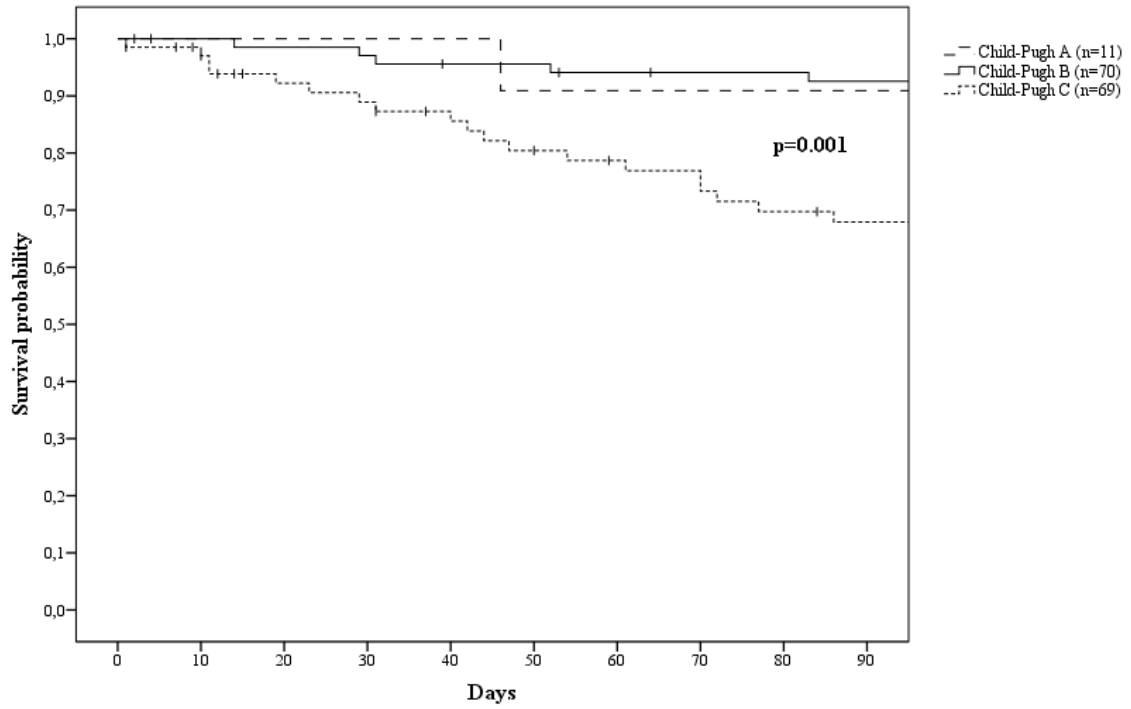


Figure 12. Survival curves for the study population according to Child-Pugh classification. The p value was calculated by the log-rank test. “|” are indicating censored patients. Reproduced from ⁽²⁵²⁾ with permission of Oxford University Press

9 Discussion

We performed a prospective, mono-centric, cohort study to investigate the prevalence of IA in patients with underlying liver cirrhosis and the performance of serum GM screening. We found a prevalence of 1.3% for probable IA. In addition, serum GM screening showed a particularly low PPV of 17% when screening all patients with decompensated liver cirrhosis or compensated liver cirrhosis plus respiratory signs and symptoms, while the PPV was increased to 50% when testing only those with clinical suspicion of IA. To our knowledge, this is the first study with a prospective design to determine the prevalence of IA in patients with underlying liver cirrhosis ⁽²⁵²⁾.

Liver diseases are not considered as a classical risk factor for developing invasive fungal diseases. Especially when it comes to IA, other well known risk factors, like hematological malignancies, HSCT, or lung transplantation have been linked to susceptibility for IA development ⁽³¹⁾. Only recently, underlying liver diseases have been reported in patients with IA even in absence of other, “classical” risk factors ^(32, 36, 38, 42, 91) raising the awareness for presence of IA in patients with liver diseases.

A particularly high number of IA cases was published previously by Gustot and colleagues in patients with underlying severe alcoholic hepatitis ⁽⁴²⁾. Alcoholic hepatitis usually occurs in patients with chronic alcohol abuse in case of recent alcohol excesses or ongoing alcohol abuse ⁽²⁵⁸⁾. Treatment of alcoholic hepatitis often requires glucocorticoid use which improves short term survival on the one hand but increases the risk of bacterial infections with associated increase in 2-month mortality on the other hand ^(259, 260). Gustot et al., however, were also able to show that a significant proportion of patients with alcoholic hepatitis develop IA. They reported a prevalence of 16% in their study ⁽⁴²⁾, which is even higher than the prevalence of IA in high risk patients on mold active prophylaxis ⁽⁴⁵⁾. A very important point when interpreting the Gustot study is, that 70% of their study population received immunosuppressive therapy with systemic glucocorticoids for alcoholic hepatitis. This may explain two very divergent findings from the Gustot study and this study. First, the prevalence in this study was much lower compared to the Gustot study (1.3% versus 16%). In this study, however, only 5.3% of the study population received glucocorticoid therapy compared to 70% of patients with severe alcoholic hepatitis. This may indicate that the main trigger for development of IA in patients with

underlying liver disease is the concomitant therapy^(42, 252). This is also reflected by the fact, that systemic high-dose glucocorticoid use (≥ 0.3 mg/kg/d prednisone equivalent for ≥ 3 weeks) is considered a host factor within the EORTC/MSG criteria⁽³³⁾ as glucocorticoids affect virtually every cell line involved in immune system resulting in cellular immunodeficiency⁽²⁶¹⁾. More than that, glucocorticoids enhance the growth of *Aspergillus fumigatus* and *Aspergillus flavus* in-vitro⁽²⁶²⁾. Second, there are significant differences in survival rates between the two studies. Patients with severe alcoholic hepatitis and IA had a devastatingly low transplant free survival of 0%⁽⁴²⁾. In this study survival reached 83%⁽²⁵²⁾. This may be based on the fact that diagnosis of IA was made retrospectively in the Gustot study, whilst in this study we performed a prospective approach that resulted in early diagnosis and proper further diagnostic work-up that led to therapeutic consequences. More than that, patients in the Gustot trial had a significantly higher model of end stage liver disease score compared to this trial (27 and 23 for patients with and without IA versus 16), which per se is associated with higher mortality rates. We cannot rule out, however, the fact that we may have missed a few patients that were admitted on weekends or holidays and died shortly after, prior to screening and study inclusion resulting in an underestimation of prevalence or mortality rate. Nevertheless, it seems unlikely as the 48h our mortality rate after admission was very low. Only two from 154 screened patients died within 48h, of whom one patient died prior to first GM serum sampling and one afterwards. The number of potentially missed patients who died due to foudroyant IA seems, therefore, to be negligible. Even the one patient who died within 48h and prior to first GM screening died due to esophageal varices bleeding and had no evidence for presence of fungal diseases on autopsy⁽²⁵²⁾.

Cases of IA have not only be reported in patients with severe alcoholic hepatitis but also in patients with end-stage cirrhosis (Child-Pugh stage C)^(38, 39, 41). Interestingly, none of the patients with Child-Pugh C liver cirrhosis in this study was diagnosed with IA, even though they accounted for 46% of all included patients. All two cases with probable IA had underlying compensated Child-Pugh A liver cirrhosis. For clinicians this may be of particular interests, as based on currently available literature, we know that critical ill patients in need for ICU admission and advanced liver cirrhosis are at risk for developing invasive mold disease. IA may be underdiagnosed in patients with Child-Pugh A or B cirrhosis as awareness of physicians for presence of IA in early and compensated stages of cirrhosis is potentially low and performance of currently available screening tools, in first

line biomarkers, in the non-neutropenic patients is limited ⁽³⁸⁾. Especially serum GM testing may be of limited value in patients without neutropenia. This is based on the fact, that neutrophils are preventing *Aspergillus* from angioinvasive growth by distinct local inflammation leading to tissue damage and tissue-invasion but not angioinvasion. Obviously, if there is no or only very limited angioinvasion only a low amount of GM will be circulating in the blood stream and may be missed when using GM as biomarker in blood samples for screening. Thus, serum GM may tend to yield false negative results in case of lack of neutropenia ^(66, 149).

In this study we also observed limited performance of serum GM screening as only one of two probable cases had a positive result. None of these two patients were neutropenic. In these patients a more targeted diagnostic approach consisting of bronchoscopy and GM determination in BALF is of additional diagnostic value as shown in our patient with a negative serum GM result (patient 2). Bronchoscopy was performed early after positive chest CT scan and prior to start of antifungal therapy in this patient. BALF-GM testing yielded a highly positive GM result (3.44) underlining the importance of timely BALF diagnostics in case of suspicion of pulmonary aspergillosis. An alternative diagnosis in this patient seems to be unlikely, as radiography and the high BALF-GM are in line with IA. BALF-GM levels ≥ 3 have been shown to yield a 100% positive predictive value for presence of IA in a mixed cohort of patients at risk for IA developing, not limited to neutropenic patients ⁽¹⁵⁴⁾. The other patient with probable IA (patient 1), however, had delayed bronchoscopy and BALF-GM testing resulted negative (0.38) after nine days after initiation of empirical mold-active antifungal treatment. Even though influence of systemic antifungal therapy on BALF-GM seems to be of less extent than on serum GM testing, the negative BALF-GM result in this patient with ongoing antifungal therapy may be explained by the reduced sensitivity of BALF-GM testing in the presence of antifungal treatment. In this case the sensitivity of BALF-GM may be reduced by about 20% (52% versus 71% in those without antifungals according to a recent study ^(101, 252)).

GM screening in the overall study cohort was highly specific but limited by a low PPV. A positive serum GM test was only associated with a 17% probability of having probable IA. Screening only patients with clinical suspicion for IA increased performance of GM screening. In particular, PPV increased to 50%, whilst there was no influence on sensitivity, specificity and NPV. Similar observations have recently been published by Duarte and colleagues for patients with hematological malignancies and ongoing mold-

active prophylaxis⁽¹⁵⁰⁾. PPV for serum GM screening in that study was only 12% when screening hematological patients under antifungal prophylaxes regardless of signs and symptoms for systemic infection, but 89.6% when only testing symptomatic patients with clinical suspicion of IA⁽¹⁵⁰⁾. A major driver of performance of biomarkers is the pretest probability of the disease which in our case is the prevalence of IA in patients with liver cirrhosis. Screening only symptomatic patients in our study would have increased the pretest probability for presence of IA from 1.3% to 15.4%, very similar to the observations of the Duarte study⁽¹⁵⁰⁾. In the setting of higher pretest probability performance of GM screening was also much more promising. Based on the findings from this study, universal GM testing for IA screening in all admitted patients with liver cirrhosis is an unreliable approach. Thus, GM testing and further diagnostic work-up should be based on clinical judgment and suspicion of IA.

Both of our patients with probable IA received voriconazole, as the recommended first line treatment for IA^(94, 220). In one patient mild side effects could be observed. The patient reported mild, temporary and reversible blurred vision. It may be that voriconazole levels were elevated temporarily resulting in increased plasma levels, leading to transient side effects. Liver function test showed elevation of AST in both patients (118-176 U/L in patient 1 and 102-314 in patient 2) shortly after initiation of voriconazole therapy but declined afterwards with ongoing therapy. A temporary trigger for the elevated AST levels may be the loading dose of voriconazole which is higher (6 mg/kg/day intravenously twice daily or 400 mg per oral twice daily) compared to maintenance dose (4 mg/kg/day intravenously twice daily or 200 mg per oral twice daily). However, dose adjustment or treatment discontinuation was not necessary in either of the patients. This is in line with previously published data, where voriconazole was associated with elevated liver enzymes in approximately 20% of treated patients but required treatment discontinuation in only 11%⁽²⁰⁶⁾. These findings were confirmed in a recently published cohort study, where elevation of liver aminotransferases >200 U/L was observed more often in patients with underlying liver diseases than in patients without, but acute liver injury was very rare (0.03%)⁽²⁶³⁾. Risk-benefit assessment of voriconazole therapy in patients with underlying liver disease is, however, very important as voriconazole is a known hepatotoxic antifungal agent and newer agents with less hepatotoxicity have become available. Isavuconazole for example, a new azole agent showed non inferiority to voriconazole in first line treatment of IA and had significantly less hepatic side effects (9% versus 16%, p=0.016)⁽¹⁰⁰⁾. As

isavuconazole is also available as intravenous solution and as oral tablet, it may be particularly interesting and promising alternative therapy approach to voriconazole in patients with underlying liver diseases. In case of oral voriconazole treatment, therapeutic drug monitoring is important to reduce side effects due to high serum levels but ensuring efficacy against *Aspergillus* spp. at the same time ⁽²⁶⁴⁾ and is therefore particularly important for patients with impaired liver function receiving voriconazole. Alternative treatment options, besides azoles, include lipAmB, and echinocandins ^(38, 39, 94, 100, 208, 252). However, lipAmB may lead to nephrotoxicity ⁽²⁶⁵⁾ which may be a reason for withholding or discontinuation of lipAmB therapy in cirrhotic patients suffering from hepatorenal syndrome. Echinocandins are also a promising alternative therapy option for treatment of IA. However, in lack of comparative trials for echinocandins versus voriconazole for first line therapy of IA, echinocandins are not recommended as first line therapy for IA but may be an proper alternative IA therapy option ⁽²⁰⁸⁾.

In this study both of the patients with IA survived. Mortality rates among patients with end stage liver diseases and IA was 53.5% in a review by Jeurissen and colleagues ⁽³⁹⁾. Several factors may explain the difference in mortality rate. First, patients in our study with IA had early stage liver cirrhosis (both Child-Pugh stage A) compared to more advanced stages of cirrhosis in several other reports. Second, due to our prospective approach, we were able to diagnose IA early in the course of disease which is critical for successful management ⁽⁸⁸⁾. Diagnosis was delayed in several previous reports and sometimes even established post-mortem ^(39, 42). Third, the majority of patients in previous reports did not receive voriconazole and in case voriconazole therapy was performed therapeutic drug monitoring was reported infrequently questioning the efficacy of antifungal therapy ⁽³⁸⁻⁴¹⁾. Last but not least, only a very low proportion of patients in this study received glucocorticoid treatment which per se is a risk for developing IA and higher mortality due to its ubiquitous effects on the immune system.

In conclusion, we found a prevalence of 1.3% of IA in patients with underlying liver cirrhosis. Targeted GM testing in case of clinical suspicion of IA may, therefore, be associated with markedly higher PPVs when compared to universal GM screening in patients with liver cirrhosis. In clinically suspected cases a negative serum GM should not withhold further diagnostic work-up to rule out IA, consisting primarily of a chest CT scan. This is crucial as diagnosis of IA early in the course of disease and rapid initiation of proper antifungal therapy improves patients' outcome.

10 Acknowledgments

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11 References

1. Ainsworth GC. Introduction to the history of mycology. Cambridge: Cambridge University Press; 1976.
2. Bossche HV, MacKenzie DWR, Cauwenbergh G. Aspergillus and Aspergillosis. New York: Springer Science+Business Media; 1988.
3. Link H. Observations in ordines plantarum naturales. Gesellschaft Naturforschender Freunde zu Berlin, Magazin. 1809;3(1):1 - 42.
4. Fresenius G. Beiträge zur Mykologie. Frankfurt am Main: Heinrich Ludwig Brönner; 1850 - 1863.
5. Schönlein JL. Zur Pathologie der Impetigenes. Archiv für Anatomie, Physiologie und wissenschaftliche Medicin. 1839:82.
6. Langenbeck B. Auffindung von Pilzen auf der Schleimhaut der Speiseröhre einer Typhus Leiche. Neue Notizen aus dem Gebiet der Natur- und Heilkunde. 1839;12(10):145 - 8.
7. Knoke M, Bernhardt H, Schwesinger G. [Early description of a pulmonary aspergillosis 1847 from Greifswald]. Mycoses. 2003;46 Suppl 1:37-41.
8. Virchow R. Beiträge zur Lehre von den beim Menschen vorkommenden pflanzlichen Parasiten. Archiv für pathologische Anatomie und Physiologie und für klinische Medizin. 1856;9:557 - 93.
9. Thoms C, Church MB. The Aspergilli. Baltimore: Williams & Wilkins; 1926.
10. Balajee SA, Houbraken J, Verweij PE, Hong SB, Yaghuchi T, Varga J, et al. Aspergillus species identification in the clinical setting. Studies in mycology. 2007;59:39-46.
11. Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Diseases. 7th ed. Philadelphia: Elsevier; 2010.
12. Klich MA, Pitt JI. A Laboratory Guide to the Common Aspergillus Species and Their Teleomorphs: Commonwealth Scientific and Industrial Research Organisation, Division of Food Processing; 1988.
13. O'Gorman CM, Fuller H, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen Aspergillus fumigatus. Nature. 2009;457(7228):471-4.

14. Gams S, Christensen M, Onions AH, Pitt JI, Samson RA. Infragenic taxa of *Aspergillus*. In: Samson RA, editor. *Advances in Penicillium and Aspergillus systematics*. New York: Springer US; 1985. p. 55-62.
15. Gastebois A, Clavaud C, Aïmanianda V, Latge JP. *Aspergillus fumigatus*: cell wall polysaccharides, their biosynthesis and organization. *Future microbiology*. 2009;4(5):583-95.
16. Abad A, Fernandez-Molina JV, Bikandi J, Ramirez A, Margareto J, Sendino J, et al. What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. *Revista iberoamericana de micologia*. 2010;27(4):155-82.
17. Free SJ. Fungal cell wall organization and biosynthesis. *Advances in genetics*. 2013;81:33-82.
18. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in Invasive Aspergillosis. *Clinical microbiology reviews*. 2009;22(3):447-65.
19. Barnes PD, Marr KA. Aspergillosis: spectrum of disease, diagnosis, and treatment. *Infectious disease clinics of North America*. 2006;20(3):545-61, vi.
20. McNeil MM, Nash SL, Hajjeh RA, Phelan MA, Conn LA, Plikaytis BD, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2001;33(5):641-7.
21. Caira M, Candoni A, Verga L, Busca A, Delia M, Nosari A, et al. Pre-chemotherapy risk factors for invasive fungal diseases: prospective analysis of 1,192 patients with newly diagnosed acute myeloid leukemia (SEIFEM 2010-a multicenter study). *Haematologica*. 2015;100(2):284-92.
22. Auberger J, Lass-Flörl C, Ulmer H, Nogler-Semenitz E, Clausen J, Gung'or E, et al. Significant alterations in the epidemiology and treatment outcome of invasive fungal infections in patients with hematological malignancies. *International journal of hematology*. 2008;88(5):508-15.
23. Perfect JR, Hachem R, Wingard JR. Update on epidemiology of and preventive strategies for invasive fungal infections in cancer patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2014;59 Suppl 5:S352-5.
24. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell

transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2010;50(8):1091-100.

25. Hoenigl M, Zollner-Schwetz I, Sill H, Linkesch W, Lass-Flörl C, Schnedl WJ, et al. Epidemiology of invasive fungal infections and rationale for antifungal therapy in patients with haematological malignancies. *Mycoses*. 2011;54(5):454-9.

26. Neofytos D, Treadway S, Ostrander D, Alonso CD, Dierberg KL, Nussenblatt V, et al. Epidemiology, outcomes, and mortality predictors of invasive mold infections among transplant recipients: a 10-year, single-center experience. *Transplant infectious disease : an official journal of the Transplantation Society*. 2013;15(3):233-42.

27. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2010;50(8):1101-11.

28. Morgan J, Wannemuehler KA, Marr KA, Hadley S, Kontoyiannis DP, Walsh TJ, et al. Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Medical mycology*. 2005;43 Suppl 1:S49-58.

29. Gregg KS, Kauffman CA. Invasive Aspergillosis: Epidemiology, Clinical Aspects, and Treatment. *Seminars in respiratory and critical care medicine*. 2015;36(5):662-72.

30. Lortholary O, Gangneux JP, Sitbon K, Lebeau B, de Monbrison F, Le Strat Y, et al. Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005-2007). *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2011;17(12):1882-9.

31. Segal BH. Aspergillosis. *The New England journal of medicine*. 2009;360(18):1870-84.

32. Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Wijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. *American journal of respiratory and critical care medicine*. 2004;170(6):621-5.

33. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group

(EORTC/MSG) Consensus Group. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;46(12):1813-21.

34. Meersseman W, Lagrou K, Maertens J, Van Wijngaerden E. Invasive aspergillosis in the intensive care unit. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2007;45(2):205-16.
35. Eigl S, Prattes J, Lackner M, Willinger B, Spiess B, Reinwald M, et al. Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. *Critical care*. 2015;19(1):178.
36. Russo A, Falcone M, Vena A, Venditti C, Mancini C, Morelli A, et al. Invasive pulmonary aspergillosis in non-neutropenic patients: analysis of a 14-month prospective clinical experience. *Journal of chemotherapy*. 2011;23(5):290-4.
37. Vandewoude K, Blot S, Benoit D, Depuydt P, Vogelaers D, Colardyn F. Invasive aspergillosis in critically ill patients: analysis of risk factors for acquisition and mortality. *Acta clinica Belgica*. 2004;59(5):251-7.
38. Falcone M, Massetti AP, Russo A, Vullo V, Venditti M. Invasive aspergillosis in patients with liver disease. *Medical mycology*. 2011;49(4):406-13.
39. Jeurissen S, Vogelaers D, Sermijn E, Van Dycke K, Geerts A, Van Vlierberghe H, et al. Invasive aspergillosis in patients with cirrhosis, a case report and review of the last 10 years. *Acta clinica Belgica*. 2013;68(5):368-75.
40. Lipke AB, Mihas AA. Non-decompensated cirrhosis as a risk factor for invasive aspergillosis: a case report and review of the immune dysfunction of cirrhosis. *The American journal of the medical sciences*. 2007;334(4):314-6.
41. Prodanovic H, Cracco C, Massard J, Barrault C, Thabut D, Duguet A, et al. Invasive pulmonary aspergillosis in patients with decompensated cirrhosis: case series. *BMC gastroenterology*. 2007;7:2.
42. Gustot T, Maillart E, Bocci M, Surin R, Trepo E, Degre D, et al. Invasive aspergillosis in patients with severe alcoholic hepatitis. *Journal of hepatology*. 2014;60(2):267-74.
43. Caira M, Girmenia C, Fadda RM, Mitra ME, Picardi M, Van Lint MT, et al. Invasive fungal infections in patients with acute myeloid leukemia and in those submitted to allogeneic hemopoietic stem cell transplant: who is at highest risk? *European journal of haematology*. 2008;81(3):242-3.
44. Garcia-Vidal C, Upton A, Kirby KA, Marr KA. Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: biological risk factors for infection

according to time after transplantation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2008;47(8):1041-50.

45. Koehler P, Hamprecht A, Bader O, Bekeredjian-Ding I, Buchheidt D, Doelken G, et al. Epidemiology of invasive aspergillosis and azole resistance in patients with acute leukaemia: the SEPIA Study. *International journal of antimicrobial agents*. 2017;49(2):218-23.

46. Mariette C, Tavernier E, Hocquet D, Huynh A, Isnard F, Legrand F, et al. Epidemiology of invasive fungal infections during induction therapy in adults with acute lymphoblastic leukemia: a GRAALL-2005 study. *Leukemia & lymphoma*. 2017;58(3):586-93.

47. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood*. 2002;100(13):4358-66.

48. Nucci M, Garnica M, Gloria AB, Lehugeur DS, Dias VC, Palma LC, et al. Invasive fungal diseases in haematopoietic cell transplant recipients and in patients with acute myeloid leukaemia or myelodysplasia in Brazil. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2013;19(8):745-51.

49. Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clinical microbiology reviews*. 1999;12(2):310-50.

50. Morgenstern DE, Gifford MA, Li LL, Doerschuk CM, Dinauer MC. Absence of respiratory burst in X-linked chronic granulomatous disease mice leads to abnormalities in both host defense and inflammatory response to *Aspergillus fumigatus*. *The Journal of experimental medicine*. 1997;185(2):207-18.

51. Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger P, Judson MA, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis--state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2003;37 Suppl 3:S225-64.

52. Ben-Ami R, Lewis RE, Kontoyiannis DP. Enemy of the (immunosuppressed) state: an update on the pathogenesis of *Aspergillus fumigatus* infection. *British journal of haematology*. 2010;150(4):406-17.

53. Cenci E, Mencacci A, Casagrande A, Mosci P, Bistoni F, Romani L. Impaired antifungal effector activity but not inflammatory cell recruitment in interleukin-6-deficient

mice with invasive pulmonary aspergillosis. *The Journal of infectious diseases*. 2001;184(5):610-7.

54. Mehrad B, Strieter RM, Standiford TJ. Role of TNF-alpha in pulmonary host defense in murine invasive aspergillosis. *Journal of immunology*. 1999;162(3):1633-40.
55. Morrison BE, Park SJ, Mooney JM, Mehrad B. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. *The Journal of clinical investigation*. 2003;112(12):1862-70.
56. Phadke AP, Mehrad B. Cytokines in host defense against *Aspergillus*: recent advances. *Medical mycology*. 2005;43 Suppl 1:S173-6.
57. Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, et al. Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nature immunology*. 2007;8(1):31-8.
58. Amanianda V, Bayry J, Bozza S, Knemeyer O, Perruccio K, Elluru SR, et al. Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature*. 2009;460(7259):1117-21.
59. Bonnett CR, Cornish EJ, Harmsen AG, Burritt JB. Early neutrophil recruitment and aggregation in the murine lung inhibit germination of *Aspergillus fumigatus* Conidia. *Infection and immunity*. 2006;74(12):6528-39.
60. Waldorf AR, Diamond RD. Neutrophil chemotactic responses induced by fresh and swollen *Rhizopus oryzae* spores and *Aspergillus fumigatus* conidia. *Infection and immunity*. 1985;48(2):458-63.
61. Philippe B, Ibrahim-Granet O, Prevost MC, Gougerot-Pocidallo MA, Sanchez Perez M, Van der Meer A, et al. Killing of *Aspergillus fumigatus* by alveolar macrophages is mediated by reactive oxidant intermediates. *Infection and immunity*. 2003;71(6):3034-42.
62. Nauseef WM. How human neutrophils kill and degrade microbes: an integrated view. *Immunological reviews*. 2007;219:88-102.
63. Gerson SL, Talbot GH, Hurwitz S, Strom BL, Lusk EJ, Cassileth PA. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Annals of internal medicine*. 1984;100(3):345-51.
64. Boss B, Neeck G, Engelhardt B, Riedel W. Influence of corticosteroids on neutrophils, lymphocytes, their subsets, and T-cell activity markers in patients with active rheumatoid arthritis, compared to healthy controls. *Annals of the New York Academy of Sciences*. 1999;876:198-200.

65. Goulding NJ, Euzger HS, Butt SK, Perretti M. Novel pathways for glucocorticoid effects on neutrophils in chronic inflammation. *Inflammation research : official journal of the European Histamine Research Society* [et al]. 1998;47 Suppl 3:S158-65.
66. Bergeron A, Porcher R, Sulahian A, de Bazelaire C, Chagnon K, Raffoux E, et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. *Blood*. 2012;119(8):1831-7; quiz 956.
67. Perkhofer S, Kehrel BE, Dierich MP, Donnelly JP, Nussbaumer W, Hofmann J, et al. Human platelets attenuate *Aspergillus* species via granule-dependent mechanisms. *The Journal of infectious diseases*. 2008;198(8):1243-6.
68. Knutsen AP, Mueller KR, Hutcheson PS, Slavin RG. T- and B-cell dysregulation of IgE synthesis in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Clinical immunology and immunopathology*. 1990;55(1):129-38.
69. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2013;43(8):850-73.
70. Stevens DA, Schwartz HJ, Lee JY, Moskovitz BL, Jerome DC, Catanzaro A, et al. A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *The New England journal of medicine*. 2000;342(11):756-62.
71. Denning DW, Cadranel J, Beigelman-Aubry C, Ader F, Chakrabarti A, Blot S, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. *The European respiratory journal*. 2016;47(1):45-68.
72. Kono Y, Tsushima K, Yamaguchi K, Kurita N, Soeda S, Fujiwara A, et al. The utility of galactomannan antigen in the bronchial washing and serum for diagnosing pulmonary aspergillosis. *Respiratory medicine*. 2013;107(7):1094-100.
73. Pagano L, Caira M, Candoni A, Offidani M, Martino B, Specchia G, et al. Invasive aspergillosis in patients with acute myeloid leukemia: a SEIFEM-2008 registry study. *Haematologica*. 2010;95(4):644-50.
74. Bernardeschi C, Foulet F, Ingen-Housz-Oro S, Ortonne N, Sitbon K, Quereux G, et al. Cutaneous Invasive Aspergillosis: Retrospective Multicenter Study of the French Invasive-Aspergillosis Registry and Literature Review. *Medicine*. 2015;94(26):e1018.

75. Kim HR, Shin JM, Lee JH, Lee HE, Im M, Lee Y, et al. Primary Cutaneous Aspergillosis after Tattoo Removal Using a 1,064-nm Q-Switched Nd:YAG Laser in an Immunocompetent Patient. *Annals of dermatology*. 2017;29(2):241-3.
76. Walsh TJ. Primary cutaneous aspergillosis--an emerging infection among immunocompromised patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1998;27(3):453-7.
77. Siddiqui AA, Shah AA, Bashir SH. Craniocerebral aspergillosis of sinonasal origin in immunocompetent patients: clinical spectrum and outcome in 25 cases. *Neurosurgery*. 2004;55(3):602-11; discussion 11-3.
78. Challa S, Uppin SG, Purohit AK. Isolated cerebral *Aspergillus* granuloma with no obvious source of infection. *Neurology India*. 2007;55(3):289-91.
79. Hoenigl M, Krause R. Antifungal therapy of aspergillosis of the central nervous system and aspergillus endophthalmitis. *Current pharmaceutical design*. 2013;19(20):3648-68.
80. Sundaram C, Umabala P, Laxmi V, Purohit AK, Prasad VS, Panigrahi M, et al. Pathology of fungal infections of the central nervous system: 17 years' experience from Southern India. *Histopathology*. 2006;49(4):396-405.
81. Boes B, Bashir R, Boes C, Hahn F, McConnell JR, McComb R. Central nervous system aspergillosis. Analysis of 26 patients. *Journal of neuroimaging : official journal of the American Society of Neuroimaging*. 1994;4(3):123-9.
82. Payot A, Garbino J, Burkhardt K, Delavelle J, Pizzolato G, Kaiser L. Primary central nervous system aspergillosis: a case report and review of the literature. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 1999;5(9):573-6.
83. Van de Wyngaert FA, Sindic CJ, Rousseau JJ, Fernandes Xavier FG, Brucher JM, Laterre EC. Spinal arachnoiditis due to aspergillus meningitis in a previously healthy patient. *Journal of neurology*. 1986;233(1):41-3.
84. Woods GL, Goldsmith JC. *Aspergillus* infection of the central nervous system in patients with acquired immunodeficiency syndrome. *Archives of neurology*. 1990;47(2):181-4.
85. Segal BH, Walsh TJ. Current approaches to diagnosis and treatment of invasive aspergillosis. *American journal of respiratory and critical care medicine*. 2006;173(7):707-17.

86. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2006;43(1):25-31.
87. Caillot D, Casasnovas O, Bernard A, Couaillier JF, Durand C, Cuisenier B, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1997;15(1):139-47.
88. von Eiff M, Roos N, Schulten R, Hesse M, Zuhlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. *Respiration; international review of thoracic diseases*. 1995;62(6):341-7.
89. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. *The Lancet infectious diseases*. 2005;5(10):609-22.
90. Ostrosky-Zeichner L. Invasive mycoses: diagnostic challenges. *The American journal of medicine*. 2012;125(1 Suppl):S14-24.
91. Prattes J, Flick H, Pruller F, Koidl C, Raggam RB, Palfner M, et al. Novel tests for diagnosis of invasive aspergillosis in patients with underlying respiratory diseases. *American journal of respiratory and critical care medicine*. 2014;190(8):922-9.
92. Hoenigl M, Salzer HJ, Raggam RB, Valentin T, Rohn A, Woelfler A, et al. Impact of galactomannan testing on the prevalence of invasive aspergillosis in patients with hematological malignancies. *Medical mycology*. 2012;50(3):266-9.
93. Hoenigl M, Valentin T, Salzer HJ, Zollner-Schwetz I, Krause R. Underestimating the real burden of invasive fungal infections in hematopoietic stem cell transplant recipients? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2010;51(2):253-4; author reply 4-5.
94. Patterson TF, Thompson GR, 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2016;63(4):e1-e60.
95. Barnes PD, Marr KA. Risks, diagnosis and outcomes of invasive fungal infections in haematopoietic stem cell transplant recipients. *British journal of haematology*. 2007;139(4):519-31.
96. Kontoyiannis DP, Sumoza D, Tarrand J, Bodey GP, Storey R, Raad, II. Significance of aspergillemia in patients with cancer: a 10-year study. *Clinical infectious*

diseases : an official publication of the Infectious Diseases Society of America. 2000;31(1):188-9.

97. Eigl S, Hoenigl M, Spiess B, Heldt S, Prattes J, Neumeister P, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. *Medical mycology*. 2016.
98. Hoenigl M, Prattes J, Spiess B, Wagner J, Pruessler F, Raggam RB, et al. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. *Journal of clinical microbiology*. 2014;52(6):2039-45.
99. Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, Meers S, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2009;49(11):1688-93.
100. Maertens JA, Raad, II, Marr KA, Patterson TF, Kontoyiannis DP, Cornely OA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. *Lancet*. 2016;387(10020):760-9.
101. Eigl S, Prattes J, Reinwald M, Thornton CR, Reischies F, Spiess B, et al. Influence of mould-active antifungal treatment on the performance of the Aspergillus-specific bronchoalveolar lavage fluid lateral-flow device test. *International journal of antimicrobial agents*. 2015;46(4):401-5.
102. Horvath JA, Dummer S. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *The American journal of medicine*. 1996;100(2):171-8.
103. Perfect JR, Cox GM, Lee JY, Kauffman CA, de Repentigny L, Chapman SW, et al. The impact of culture isolation of Aspergillus species: a hospital-based survey of aspergillosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2001;33(11):1824-33.
104. De Carolis E, Posteraro B, Lass-Florl C, Vella A, Florio AR, Torelli R, et al. Species identification of Aspergillus, Fusarium and Mucorales with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2012;18(5):475-84.

105. Alexander BD. Diagnosis of fungal infection: new technologies for the mycology laboratory. *Transplant infectious disease : an official journal of the Transplantation Society*. 2002;4 Suppl 3:32-7.
106. Vehreschild JJ, Ruping MJ, Steinbach A, Cornely OA. Diagnosis and treatment of fungal infections in allogeneic stem cell and solid organ transplant recipients. *Expert opinion on pharmacotherapy*. 2010;11(1):95-113.
107. Patel IJ, Davidson JC, Nikolic B, Salazar GM, Schwartzberg MS, Walker TG, et al. Consensus guidelines for periprocedural management of coagulation status and hemostasis risk in percutaneous image-guided interventions. *Journal of vascular and interventional radiology : JVIR*. 2012;23(6):727-36.
108. Labarca JA, Wagar EA, Grasmick AE, Kokkinos HM, Bruckner DA. Critical evaluation of 4-week incubation for fungal cultures: is the fourth week useful? *Journal of clinical microbiology*. 1998;36(12):3683-5.
109. Hauggaard A, Ellis M, Ekelund L. Early chest radiography and CT in the diagnosis, management and outcome of invasive pulmonary aspergillosis. *Acta radiologica*. 2002;43(3):292-8.
110. Becker MJ, Lugtenburg EJ, Cornelissen JJ, Van Der Schee C, Hoogsteden HC, De Marie S. Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in haematological patients at risk for invasive pulmonary aspergillosis. *British journal of haematology*. 2003;121(3):448-57.
111. Weisser M, Rausch C, Droll A, Simcock M, Sendi P, Steffen I, et al. Galactomannan does not precede major signs on a pulmonary computerized tomographic scan suggestive of invasive aspergillosis in patients with hematological malignancies. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2005;41(8):1143-9.
112. Busca A, Locatelli F, Barbui A, Limerutti G, Serra R, Libertucci D, et al. Usefulness of sequential *Aspergillus* galactomannan antigen detection combined with early radiologic evaluation for diagnosis of invasive pulmonary aspergillosis in patients undergoing allogeneic stem cell transplantation. *Transplantation proceedings*. 2006;38(5):1610-3.
113. Maertens J, Meersseman W, Van Bleyenbergh P. New therapies for fungal pneumonia. *Current opinion in infectious diseases*. 2009;22(2):183-90.
114. Brodoefel H, Vogel M, Hebart H, Einsele H, Vonthein R, Claussen C, et al. Long-term CT follow-up in 40 non-HIV immunocompromised patients with invasive pulmonary

aspergillosis: kinetics of CT morphology and correlation with clinical findings and outcome. *AJR American journal of roentgenology*. 2006;187(2):404-13.

115. Caillot D, Couaillier JF, Bernard A, Casasnovas O, Denning DW, Mannone L, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2001;19(1):253-9.

116. Greene RE, Schlamm HT, Oestmann JW, Stark P, Durand C, Lortholary O, et al. Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2007;44(3):373-9.

117. Horger M, Hebart H, Einsele H, Lengerke C, Claussen CD, Vonthein R, et al. Initial CT manifestations of invasive pulmonary aspergillosis in 45 non-HIV immunocompromised patients: association with patient outcome? *European journal of radiology*. 2005;55(3):437-44.

118. Sassi C, Stanzani M, Lewis RE. Computerized tomographic pulmonary angiography discriminates invasive mould disease of the lung from lymphoma. *British journal of haematology*. 2015;169(4):462.

119. Stanzani M, Sassi C, Lewis RE, Tolomelli G, Bazzocchi A, Cavo M, et al. High resolution computed tomography angiography improves the radiographic diagnosis of invasive mold disease in patients with hematological malignancies. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2015;60(11):1603-10.

120. Poelmans J, Hillen A, Vanherp L, Govaerts K, Maertens J, Dresselaers T, et al. Longitudinal, in vivo assessment of invasive pulmonary aspergillosis in mice by computed tomography and magnetic resonance imaging. *Laboratory investigation; a journal of technical methods and pathology*. 2016;96(6):692-704.

121. Vehreschild JJ, Heussel CP, Groll AH, Vehreschild MJ, Silling G, Wurthwein G, et al. Serial assessment of pulmonary lesion volume by computed tomography allows survival prediction in invasive pulmonary aspergillosis. *European radiology*. 2017.

122. Blum U, Windfuhr M, Buitrago-Tellez C, Sigmund G, Herbst EW, Langer M. Invasive pulmonary aspergillosis. MRI, CT, and plain radiographic findings and their contribution for early diagnosis. *Chest*. 1994;106(4):1156-61.

123. Siddiqui AA, Bashir SH, Ali Shah A, Sajjad Z, Ahmed N, Jooma R, et al. Diagnostic MR imaging features of craniocerebral Aspergillosis of sino-nasal origin in immunocompetent patients. *Acta neurochirurgica*. 2006;148(2):155-66; discussion 66.
124. Starkey J, Moritani T, Kirby P. MRI of CNS fungal infections: review of aspergillosis to histoplasmosis and everything in between. *Clinical neuroradiology*. 2014;24(3):217-30.
125. Rolle AM, Hasenberg M, Thornton CR, Solouk-Saran D, Mann L, Weski J, et al. ImmunoPET/MR imaging allows specific detection of *Aspergillus fumigatus* lung infection in vivo. *Proc Natl Acad Sci U S A*. 2016;113(8):E1026-33.
126. Petrik M, Franssen GM, Haas H, Laverman P, Hortnagl C, Schrettl M, et al. Preclinical evaluation of two ⁶⁸Ga-siderophores as potential radiopharmaceuticals for *Aspergillus fumigatus* infection imaging. *European journal of nuclear medicine and molecular imaging*. 2012;39(7):1175-83.
127. Leeflang MM, Debets-Ossenkopp YJ, Visser CE, Scholten RJ, Hooft L, Bijlmer HA, et al. Galactomannan detection for invasive aspergillosis in immunocompromized patients. *The Cochrane database of systematic reviews*. 2008(4):CD007394.
128. Guo YL, Chen YQ, Wang K, Qin SM, Wu C, Kong JL. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate metaanalysis and systematic review. *Chest*. 2010;138(4):817-24.
129. Duettmann W, Koidl C, Troppan K, Seeber K, Buzina W, Wolfler A, et al. Serum and urine galactomannan testing for screening in patients with hematological malignancies. *Medical mycology*. 2014;52(6):647-52.
130. Chong GM, Maertens JA, Lagrou K, Driessen GJ, Cornelissen JJ, Rijnders BJ. Diagnostic Performance of Galactomannan Antigen Testing in Cerebrospinal Fluid. *Journal of clinical microbiology*. 2016;54(2):428-31.
131. Tortorano AM, Esposto MC, Prigitano A, Grancini A, Ossi C, Cavanna C, et al. Cross-reactivity of *Fusarium* spp. in the *Aspergillus* Galactomannan enzyme-linked immunosorbent assay. *Journal of clinical microbiology*. 2012;50(3):1051-3.
132. Huang YT, Hung CC, Hsueh PR. *Aspergillus* galactomannan antigenemia in penicilliosis marneffeii. *Aids*. 2007;21(14):1990-1.
133. Wheat LJ, Hackett E, Durkin M, Connolly P, Petraitiene R, Walsh TJ, et al. Histoplasmosis-associated cross-reactivity in the BioRad Platelia *Aspergillus* enzyme immunoassay. *Clinical and vaccine immunology : CVI*. 2007;14(5):638-40.

134. Valentin T, Neumeister P, Pichler M, Rohn A, Koidl C, Haas D, et al. Disseminated *Geosmithia argillacea* infection in a patient with gastrointestinal GvHD. Bone marrow transplantation. 2012;47(5):734-6.
135. Martin-Rabadan P, Gijon P, Alonso Fernandez R, Ballesteros M, Anguita J, Bouza E. False-positive *Aspergillus* antigenemia due to blood product conditioning fluids. *Clinical infectious diseases* : an official publication of the Infectious Diseases Society of America. 2012;55(4):e22-7.
136. Petraitiene R, Petraitis V, Witt JR, 3rd, Durkin MM, Bacher JD, Wheat LJ, et al. Galactomannan antigenemia after infusion of gluconate-containing Plasma-Lyte. *Journal of clinical microbiology*. 2011;49(12):4330-2.
137. Ansorg R, van den Boom R, Rath PM. Detection of *Aspergillus* galactomannan antigen in foods and antibiotics. *Mycoses*. 1997;40(9-10):353-7.
138. Sulahian A, Touratier S, Ribaud P. False positive test for *aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. *The New England journal of medicine*. 2003;349(24):2366-7.
139. Mikulska M, Furfaro E, Del Bono V, Raiola AM, Ratto S, Bacigalupo A, et al. Piperacillin/tazobactam (Tazocin) seems to be no longer responsible for false-positive results of the galactomannan assay. *The Journal of antimicrobial chemotherapy*. 2012;67(7):1746-8.
140. De Vlieger G, Lagrou K, Maertens J, Verbeken E, Meersseman W, Van Wijngaerden E. Beta-D-glucan detection as a diagnostic test for invasive aspergillosis in immunocompromised critically ill patients with symptoms of respiratory infection: an autopsy-based study. *Journal of clinical microbiology*. 2011;49(11):3783-7.
141. Prattes J, Koidl C, Eigl S, Krause R, Hoenigl M. Bronchoalveolar lavage fluid sample pretreatment with Sputasol((R)) significantly reduces galactomannan levels. *The Journal of infection*. 2015;70(5):541-3.
142. Gils S, Maertens J, Lagrou K. Pretreatment of Bronchoalveolar Lavage Fluid Samples with SLsolution Leads to False-Negative *Aspergillus* Galactomannan Levels. *Journal of clinical microbiology*. 2016;54(4):1171.
143. Hsu JL, Ruoss SJ, Bower ND, Lin M, Holodniy M, Stevens DA. Diagnosing invasive fungal disease in critically ill patients. *Critical reviews in microbiology*. 2011;37(4):277-312.
144. Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S, et al. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal

diseases in leukemic patients and hematopoietic SCT recipients. Bone marrow transplantation. 2012;47(6):846-54.

145. Maertens JA, Klont R, Masson C, Theunissen K, Meersseman W, Lagrou K, et al. Optimization of the cutoff value for the Aspergillus double-sandwich enzyme immunoassay. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2007;44(10):1329-36.

146. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2006;42(10):1417-27.

147. Zhou W, Li H, Zhang Y, Huang M, He Q, Li P, et al. Diagnostic Value of the Galactomannan Antigen Test in Serum and Bronchoalveolar Lavage Fluid Samples from Patients with Non-neutropenic Invasive Pulmonary Aspergillosis. *Journal of clinical microbiology.* 2017.

148. Husain S, Clancy CJ, Nguyen MH, Swartzentruber S, Leather H, LeMonte AM, et al. Performance characteristics of the platelia Aspergillus enzyme immunoassay for detection of Aspergillus galactomannan antigen in bronchoalveolar lavage fluid. *Clinical and vaccine immunology : CVI.* 2008;15(12):1760-3.

149. Cordonnier C, Botterel F, Ben Amor R, Pautas C, Maury S, Kuentz M, et al. Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2009;15(1):81-6.

150. Duarte RF, Sanchez-Ortega I, Cuesta I, Arnan M, Patino B, Fernandez de Sevilla A, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2014;59(12):1696-702.

151. Cornely OA. Galactomannan testing during mold-active prophylaxis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2014;59(12):1703-4.

152. Marr KA, Laverdiere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2005;40(12):1762-9.

153. Zou M, Tang L, Zhao S, Zhao Z, Chen L, Chen P, et al. Systematic review and meta-analysis of detecting galactomannan in bronchoalveolar lavage fluid for diagnosing invasive aspergillosis. *PLoS One*. 2012;7(8):e43347.
154. D'Haese J, Theunissen K, Vermeulen E, Schoemans H, De Vlieger G, Lammertijn L, et al. Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. *Journal of clinical microbiology*. 2012;50(4):1258-63.
155. Nguyen MH, Leather H, Clancy CJ, Cline C, Jantz MA, Kulkarni V, et al. Galactomannan testing in bronchoalveolar lavage fluid facilitates the diagnosis of invasive pulmonary aspergillosis in patients with hematologic malignancies and stem cell transplant recipients. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2011;17(7):1043-50.
156. Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vanderschueren S, et al. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *American journal of respiratory and critical care medicine*. 2008;177(1):27-34.
157. He H, Ding L, Sun B, Li F, Zhan Q. Role of galactomannan determinations in bronchoalveolar lavage fluid samples from critically ill patients with chronic obstructive pulmonary disease for the diagnosis of invasive pulmonary aspergillosis: a prospective study. *Critical care*. 2012;16(4):R138.
158. Miyazaki T, Kohno S, Mitsutake K, Maesaki S, Tanaka K, Ishikawa N, et al. Plasma (1->3)-beta-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *Journal of clinical microbiology*. 1995;33(12):3115-8.
159. Odabasi Z, Paetznick VL, Rodriguez JR, Chen E, McGinnis MR, Ostrosky-Zeichner L. Differences in beta-glucan levels in culture supernatants of a variety of fungi. *Medical mycology*. 2006;44(3):267-72.
160. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2011;52(6):750-70.
161. Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, et al. beta-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clinical*

infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54(5):633-43.

162. Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2004;39(2):199-205.

163. Reischies FM, Prattes J, Woelfler A, Eigl S, Hoenigl M. Diagnostic performance of 1,3-beta-D-glucan serum screening in patients receiving hematopoietic stem cell transplantation. *Transplant infectious disease : an official journal of the Transplantation Society*. 2016;18(3):466-70.

164. Prattes J, Hoenigl M, Rabensteiner J, Raggam RB, Pruessler F, Zollner-Schwetz I, et al. Serum 1,3-beta-d-glucan for antifungal treatment stratification at the intensive care unit and the influence of surgery. *Mycoses*. 2014.

165. Posteraro B, Tumbarello M, De Pascale G, Liberto E, Vallecoccia MS, De Carolis E, et al. (1,3)-beta-d-Glucan-based antifungal treatment in critically ill adults at high risk of candidaemia: an observational study. *The Journal of antimicrobial chemotherapy*. 2016;71(8):2262-9.

166. Acosta J, Catalan M, del Palacio-Perez-Medel A, Lora D, Montejo JC, Cuetara MS, et al. A prospective comparison of galactomannan in bronchoalveolar lavage fluid for the diagnosis of pulmonary invasive aspergillosis in medical patients under intensive care: comparison with the diagnostic performance of galactomannan and of (1--> 3)-beta-d-glucan chromogenic assay in serum samples. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2011;17(7):1053-60.

167. Hachem RY, Kontoyiannis DP, Chemaly RF, Jiang Y, Reitzel R, Raad I. Utility of galactomannan enzyme immunoassay and (1,3) beta-D-glucan in diagnosis of invasive fungal infections: low sensitivity for *Aspergillus fumigatus* infection in hematologic malignancy patients. *Journal of clinical microbiology*. 2009;47(1):129-33.

168. Kami M, Tanaka Y, Kanda Y, Ogawa S, Masumoto T, Ohtomo K, et al. Computed tomographic scan of the chest, latex agglutination test and plasma (1AE3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. *Haematologica*. 2000;85(7):745-52.

169. Sulahian A, Porcher R, Bergeron A, Touratier S, Raffoux E, Menotti J, et al. Use and limits of (1-3)-beta-d-glucan assay (Fungitell), compared to galactomannan determination (Platelia Aspergillus), for diagnosis of invasive aspergillosis. *Journal of clinical microbiology*. 2014;52(7):2328-33.
170. Rose SR, Vallabhajosyula S, Velez MG, Fedorko DP, VanRaden MJ, Gea-Banacloche JC, et al. The utility of bronchoalveolar lavage beta-D-glucan testing for the diagnosis of invasive fungal infections. *The Journal of infection*. 2014;69(3):278-83.
171. Mutschlechner W, Risslegger B, Willinger B, Hoenigl M, Bucher B, Eschertzhuber S, et al. Bronchoalveolar Lavage Fluid (1,3)beta-D-Glucan for the Diagnosis of Invasive Fungal Infections in Solid Organ Transplantation: A Prospective Multicenter Study. *Transplantation*. 2015;99(9):e140-4.
172. Krause R, Halwachs B, Thallinger GG, Klymiuk I, Gorkiewicz G, Hoenigl M, et al. Characterisation of Candida within the Mycobiome/Microbiome of the Lower Respiratory Tract of ICU Patients. *PLoS One*. 2016;11(5):e0155033.
173. Krause R, Moissl-Eichinger C, Halwachs B, Gorkiewicz G, Berg G, Valentin T, et al. Mycobiome in the Lower Respiratory Tract - A Clinical Perspective. *Frontiers in microbiology*. 2016;7:2169.
174. Meersseman W, Lagrou K, Spriet I, Maertens J, Verbeken E, Peetermans WE, et al. Significance of the isolation of Candida species from airway samples in critically ill patients: a prospective, autopsy study. *Intensive care medicine*. 2009;35(9):1526-31.
175. Reischies FM, Prattes J, Pruller F, Eigl S, List A, Wolfler A, et al. Prognostic potential of 1,3-beta-d-glucan levels in bronchoalveolar lavage fluid samples. *The Journal of infection*. 2016;72(1):29-35.
176. Ellis M, Al-Ramadi B, Finkelman M, Hedstrom U, Kristensen J, Ali-Zadeh H, et al. Assessment of the clinical utility of serial beta-D-glucan concentrations in patients with persistent neutropenic fever. *Journal of medical microbiology*. 2008;57(Pt 3):287-95.
177. Held J, Kohlberger I, Rappold E, Busse Grawitz A, Hacker G. Comparison of (1->3)-beta-D-glucan, mannan/anti-mannan antibodies, and Cand-Tec Candida antigen as serum biomarkers for candidemia. *Journal of clinical microbiology*. 2013;51(4):1158-64.
178. Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR. Reactivity of (1->3)-beta-d-glucan assay with commonly used intravenous antimicrobials. *Antimicrobial agents and chemotherapy*. 2006;50(10):3450-3.

179. Otto GP, Ludewig K, Jacobsen ID, Schaarschmidt B, Hube B, Bauer M. Limitation of (1-->3)-beta-D-glucan monitoring in major elective surgery involving cardiopulmonary bypass. *Critical care*. 2013;17(3):437.
180. Posteraro B, De Pascale G, Tumbarello M, Torelli R, Pennisi MA, Bello G, et al. Early diagnosis of candidemia in intensive care unit patients with sepsis: a prospective comparison of (1-->3)-beta-D-glucan assay, Candida score, and colonization index. *Critical care*. 2011;15(5):R249.
181. Usami M, Ohata A, Horiuchi T, Nagasawa K, Wakabayashi T, Tanaka S. Positive (1-->3)-beta-D-glucan in blood components and release of (1-->3)-beta-D-glucan from depth-type membrane filters for blood processing. *Transfusion*. 2002;42(9):1189-95.
182. Prattes J, Schilcher G, Krause R. Reliability of serum 1,3-beta-d-glucan assay in patients undergoing renal replacement therapy: a review of the literature. *Mycoses*. 2015;58(1):4-9.
183. Prattes J, Schneditz D, Pruller F, Jandl E, Sauseng N, Hoenigl M, et al. 1,3-ss-d-Glucan testing is highly specific in patients undergoing dialysis treatment. *The Journal of infection*. 2017;74(1):72-80.
184. Orasch T, Prattes J, Faserl K, Eigl S, Duttmann W, Lindner H, et al. Bronchoalveolar lavage triacetylfusarinine C (TAFC) determination for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies. *The Journal of infection*. 2017.
185. Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. *Clinical and vaccine immunology : CVI*. 2008;15(7):1095-105.
186. Prattes J, Lackner M, Eigl S, Reischies F, Raggam RB, Koidl C, et al. Diagnostic accuracy of the Aspergillus-specific bronchoalveolar lavage lateral-flow assay in haematological malignancy patients. *Mycoses*. 2015;58(8):461-9.
187. Cummings JR, Jamison GR, Boudreaux JW, Howles MJ, Walsh TJ, Hayden RT. Cross-reactivity of non-Aspergillus fungal species in the Aspergillus galactomannan enzyme immunoassay. *Diagnostic microbiology and infectious disease*. 2007;59(1):113-5.
188. Thornton C, Johnson G, Agrawal S. Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. *Journal of visualized experiments : JoVE*. 2012(61).
189. Reinwald M, Spiess B, Heinz WJ, Vehreschild JJ, Lass-Florl C, Kiehl M, et al. Diagnosing pulmonary aspergillosis in patients with hematological malignancies: a

multicenter prospective evaluation of an *Aspergillus* PCR assay and a galactomannan ELISA in bronchoalveolar lavage samples. *European journal of haematology*. 2012;89(2):120-7.

190. Wiederhold NP, Najvar LK, Bocanegra R, Kirkpatrick WR, Patterson TF, Thornton CR. Interlaboratory and interstudy reproducibility of a novel lateral-flow device and influence of antifungal therapy on detection of invasive pulmonary aspergillosis. *Journal of clinical microbiology*. 2013;51(2):459-65.

191. Hoenigl M, Koidl C, Duettmann W, Seeber K, Wagner J, Buzina W, et al. Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis diagnosis in haematological malignancy and solid organ transplant patients. *The Journal of infection*. 2012;65(6):588-91.

192. Willinger B, Lackner M, Lass-Flörl C, Prattes J, Posch V, Selitsch B, et al. Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis in solid organ transplant patients: a semipropective multicenter study. *Transplantation*. 2014;98(8):898-902.

193. Mery A, Sendid B, Francois N, Cornu M, Poissy J, Guerardel Y, et al. Application of Mass Spectrometry Technology to Early Diagnosis of Invasive Fungal Infections. *Journal of clinical microbiology*. 2016;54(11):2786-97.

194. Maschmeyer G, Patterson TF. Our 2014 approach to breakthrough invasive fungal infections. *Mycoses*. 2014;57(11):645-51.

195. Tissot F, Agrawal S, Pagano L, Petrikos G, Groll AH, Skiada A, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica*. 2017;102(3):433-44.

196. Barrett JP, Vardulaki KA, Conlon C, Cooke J, Daza-Ramirez P, Evans EG, et al. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. *Clinical therapeutics*. 2003;25(5):1295-320.

197. Anderson TM, Clay MC, Cioffi AG, Diaz KA, Hisao GS, Tuttle MD, et al. Amphotericin forms an extramembranous and fungicidal sterol sponge. *Nature chemical biology*. 2014;10(5):400-6.

198. Bowden R, Chandrasekar P, White MH, Li X, Pietrelli L, Gurwith M, et al. A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients.

Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002;35(4):359-66.

199. Cornely OA, Maertens J, Bresnik M, Ebrahimi R, Ullmann AJ, Bouza E, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2007;44(10):1289-97.

200. Lanternier F, Poiree S, Elie C, Garcia-Hermoso D, Bakouboula P, Sitbon K, et al. Prospective pilot study of high-dose (10 mg/kg/day) liposomal amphotericin B (L-AMB) for the initial treatment of mucormycosis. *The Journal of antimicrobial chemotherapy.* 2015;70(11):3116-23.

201. Kirkpatrick WR, Perea S, Coco BJ, Patterson TF. Efficacy of caspofungin alone and in combination with voriconazole in a Guinea pig model of invasive aspergillosis. *Antimicrobial agents and chemotherapy.* 2002;46(8):2564-8.

202. Martin-Vicente A, Capilla J, Guarro J. Synergistic effect of anidulafungin combined with posaconazole in experimental aspergillosis. *Medical mycology.* 2017;55(4):457-60.

203. Petraitis V, Petraitiene R, Groll AH, Roussillon K, Hemmings M, Lyman CA, et al. Comparative antifungal activities and plasma pharmacokinetics of micafungin (FK463) against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropenic rabbits. *Antimicrobial agents and chemotherapy.* 2002;46(6):1857-69.

204. Bowman JC, Hicks PS, Kurtz MB, Rosen H, Schmatz DM, Liberator PA, et al. The antifungal echinocandin caspofungin acetate kills growing cells of *Aspergillus fumigatus* in vitro. *Antimicrobial agents and chemotherapy.* 2002;46(9):3001-12.

205. Kyriakidis I, Tragiannidis A, Munchen S, Groll AH. Clinical hepatotoxicity associated with antifungal agents. *Expert opinion on drug safety.* 2017;16(2):149-65.

206. Wang JL, Chang CH, Young-Xu Y, Chan KA. Systematic review and meta-analysis of the tolerability and hepatotoxicity of antifungals in empirical and definitive therapy for invasive fungal infection. *Antimicrobial agents and chemotherapy.* 2010;54(6):2409-19.

207. Ruhnke M, Rickerts V, Cornely OA, Buchheidt D, Glockner A, Heinz W, et al. Diagnosis and therapy of *Candida* infections: joint recommendations of the German Speaking Mycological Society and the Paul-Ehrlich-Society for Chemotherapy. *Mycoses.* 2011;54(4):279-310.

208. Heinz WJ, Buchheidt D, Ullmann AJ. Clinical evidence for caspofungin monotherapy in the first-line and salvage therapy of invasive *Aspergillus* infections. *Mycoses*. 2016.
209. Zonios DI, Bennett JE. Update on azole antifungals. *Seminars in respiratory and critical care medicine*. 2008;29(2):198-210.
210. Clancy CJ, Nguyen MH. In vitro efficacy and fungicidal activity of voriconazole against *Aspergillus* and *Fusarium* species. *European journal of clinical microbiology & infectious diseases* : official publication of the European Society of Clinical Microbiology. 1998;17(8):573-5.
211. Boucher HW, Groll AH, Chiou CC, Walsh TJ. Newer systemic antifungal agents : pharmacokinetics, safety and efficacy. *Drugs*. 2004;64(18):1997-2020.
212. Hyland R, Jones BC, Smith DA. Identification of the cytochrome P450 enzymes involved in the N-oxidation of voriconazole. *Drug metabolism and disposition: the biological fate of chemicals*. 2003;31(5):540-7.
213. Kim SH, Lee DG, Kwon JC, Lee HJ, Cho SY, Park C, et al. Clinical Impact of Cytochrome P450 2C19 Genotype on the Treatment of Invasive Aspergillosis under Routine Therapeutic Drug Monitoring of Voriconazole in a Korean Population. *Infection & chemotherapy*. 2013;45(4):406-14.
214. Zonios D, Yamazaki H, Murayama N, Natarajan V, Palmore T, Childs R, et al. Voriconazole metabolism, toxicity, and the effect of cytochrome P450 2C19 genotype. *The Journal of infectious diseases*. 2014;209(12):1941-8.
215. Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. *Clinical infectious diseases* : an official publication of the Infectious Diseases Society of America. 2003;36(5):630-7.
216. Lazarus HM, Blumer JL, Yanovich S, Schlamm H, Romero A. Safety and pharmacokinetics of oral voriconazole in patients at risk of fungal infection: a dose escalation study. *Journal of clinical pharmacology*. 2002;42(4):395-402.
217. Purkins L, Wood N, Ghahramani P, Greenhalgh K, Allen MJ, Kleinermans D. Pharmacokinetics and safety of voriconazole following intravenous- to oral-dose escalation regimens. *Antimicrobial agents and chemotherapy*. 2002;46(8):2546-53.
218. Walsh TJ, Lutsar I, Driscoll T, Dupont B, Roden M, Ghahramani P, et al. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *The Pediatric infectious disease journal*. 2002;21(3):240-8.

219. Eiden C, Peyriere H, Cociglio M, Djezzar S, Hansel S, Blayac JP, et al. Adverse effects of voriconazole: analysis of the French Pharmacovigilance Database. *The Annals of pharmacotherapy*. 2007;41(5):755-63.
220. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *The New England journal of medicine*. 2002;347(6):408-15.
221. Boyd AE, Modi S, Howard SJ, Moore CB, Keevil BG, Denning DW. Adverse reactions to voriconazole. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2004;39(8):1241-4.
222. den Hollander JG, van Arkel C, Rijnders BJ, Lugtenburg PJ, de Marie S, Levin MD. Incidence of voriconazole hepatotoxicity during intravenous and oral treatment for invasive fungal infections. *The Journal of antimicrobial chemotherapy*. 2006;57(6):1248-50.
223. Luong ML, Hosseini-Moghaddam SM, Singer LG, Chaparro C, Azad S, Lazar N, et al. Risk factors for voriconazole hepatotoxicity at 12 weeks in lung transplant recipients. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2012;12(7):1929-35.
224. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2002;34(5):563-71.
225. Schwartz S, Ruhnke M, Ribaud P, Corey L, Driscoll T, Cornely OA, et al. Improved outcome in central nervous system aspergillosis, using voriconazole treatment. *Blood*. 2005;106(8):2641-5.
226. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *The New England journal of medicine*. 2007;356(4):348-59.
227. Sanchez-Ortega I, Patino B, Arnan M, Peralta T, Parody R, Gudiol C, et al. Clinical efficacy and safety of primary antifungal prophylaxis with posaconazole vs itraconazole in allogeneic blood and marrow transplantation. *Bone marrow transplantation*. 2011;46(5):733-9.
228. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *The New England journal of medicine*. 2007;356(4):335-47.

229. Prattes J, Duettmann W, Hoenigl M. Posaconazole Plasma Concentrations on Days Three to Five Predict Steady-State Levels. *Antimicrobial agents and chemotherapy*. 2016;60(9):5595-9.
230. Miceli MH, Kauffman CA. Isavuconazole: A New Broad-Spectrum Triazole Antifungal Agent. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2015;61(10):1558-65.
231. Caillot D, Thiebaut A, Herbrecht R, de Botton S, Pigneux A, Bernard F, et al. Liposomal amphotericin B in combination with caspofungin for invasive aspergillosis in patients with hematologic malignancies: a randomized pilot study (Combistrat trial). *Cancer*. 2007;110(12):2740-6.
232. Marr KA, Schlamm HT, Herbrecht R, Rottinghaus ST, Bow EJ, Cornely OA, et al. Combination antifungal therapy for invasive aspergillosis: a randomized trial. *Annals of internal medicine*. 2015;162(2):81-9.
233. Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *Journal of hepatology*. 2014;61(6):1385-96.
234. Smedsrod B, Pertoft H, Gustafson S, Laurent TC. Scavenger functions of the liver endothelial cell. *The Biochemical journal*. 1990;266(2):313-27.
235. Willekens FL, Werre JM, Kruijt JK, Roerdinkholder-Stoelwinder B, Groenen-Dopp YA, van den Bos AG, et al. Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. *Blood*. 2005;105(5):2141-5.
236. Gao B, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. *Hepatology*. 2008;47(2):729-36.
237. Helmy KY, Katschke KJ, Jr., Gorgani NN, Kljavin NM, Elliott JM, Diehl L, et al. CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell*. 2006;124(5):915-27.
238. Jenne CN, Kubes P. Immune surveillance by the liver. *Nature immunology*. 2013;14(10):996-1006.
239. Fiuza C, Salcedo M, Clemente G, Tellado JM. In vivo neutrophil dysfunction in cirrhotic patients with advanced liver disease. *The Journal of infectious diseases*. 2000;182(2):526-33.
240. Gomez F, Ruiz P, Schreiber AD. Impaired function of macrophage Fc gamma receptors and bacterial infection in alcoholic cirrhosis. *The New England journal of medicine*. 1994;331(17):1122-8.

241. Ono Y, Watanabe T, Matsumoto K, Ito T, Kunii O, Goldstein E. Opsonophagocytic dysfunction in patients with liver cirrhosis and low responses to tumor necrosis factor- α and lipopolysaccharide in patients' blood. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2004;10(4):200-7.
242. Rajkovic IA, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology*. 1986;6(2):252-62.
243. Fiuza C, Salcedo M, Clemente G, Tellado JM. Granulocyte colony-stimulating factor improves deficient in vitro neutrophil transendothelial migration in patients with advanced liver disease. *Clinical and diagnostic laboratory immunology*. 2002;9(2):433-9.
244. Tritto G, Bechlis Z, Stadlbauer V, Davies N, Frances R, Shah N, et al. Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *Journal of hepatology*. 2011;55(3):574-81.
245. Wong F, Bernardi M, Balk R, Christman B, Moreau R, Garcia-Tsao G, et al. Sepsis in cirrhosis: report on the 7th meeting of the International Ascites Club. *Gut*. 2005;54(5):718-25.
246. Cervoni JP, Thevenot T, Weil D, Muel E, Barbot O, Sheppard F, et al. C-reactive protein predicts short-term mortality in patients with cirrhosis. *Journal of hepatology*. 2012;56(6):1299-304.
247. Fischer J, Silva TE, Soares ESPE, Colombo BS, Silva MC, Wildner LM, et al. From stable disease to acute-on-chronic liver failure: Circulating cytokines are related to prognosis in different stages of cirrhosis. *Cytokine*. 2017;91:162-9.
248. Pieri G, Agarwal B, Burroughs AK. C-reactive protein and bacterial infection in cirrhosis. *Annals of gastroenterology*. 2014;27(2):113-20.
249. Chamilos G, Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). *Haematologica*. 2006;91(7):986-9.
250. Burton EC, Nemetz PN. Medical error and outcomes measures: where have all the autopsies gone? *MedGenMed : Medscape general medicine*. 2000;2(2):E8.
251. Reichenberger F, Habicht J, Matt P, Frei R, Soler M, Bolliger CT, et al. Diagnostic yield of bronchoscopy in histologically proven invasive pulmonary aspergillosis. *Bone marrow transplantation*. 1999;24(11):1195-9.

252. Prattes J, Hoenigl M, Krause R, Buzina W, Valentin T, Reischies F, et al. Invasive aspergillosis in patients with underlying liver cirrhosis: a prospective cohort study. *Medical mycology*. 2017.
253. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *Journal of hepatology*. 2006;44(1):217-31.
254. Asrani SK, Kamath PS. Natural history of cirrhosis. *Current gastroenterology reports*. 2013;15(2):308.
255. Cornillet A, Camus C, Nimubona S, Gandemer V, Tattevin P, Belleguic C, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2006;43(5):577-84.
256. Hoenigl M, Strenger V, Buzina W, Valentin T, Koidl C, Wolfler A, et al. European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) host factors and invasive fungal infections in patients with haematological malignancies. *The Journal of antimicrobial chemotherapy*. 2012;67(8):2029-33.
257. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in medicine*. 1998;17(8):857-72.
258. Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. *The New England journal of medicine*. 2009;360(26):2758-69.
259. Louvet A, Wartel F, Castel H, Dharancy S, Hollebecque A, Canva-Delcambre V, et al. Infection in patients with severe alcoholic hepatitis treated with steroids: early response to therapy is the key factor. *Gastroenterology*. 2009;137(2):541-8.
260. Mathurin P, O'Grady J, Carithers RL, Phillips M, Louvet A, Mendenhall CL, et al. Corticosteroids improve short-term survival in patients with severe alcoholic hepatitis: meta-analysis of individual patient data. *Gut*. 2011;60(2):255-60.
261. Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. *Lancet*. 2003;362(9398):1828-38.
262. Ng TT, Robson GD, Denning DW. Hydrocortisone-enhanced growth of *Aspergillus* spp.: implications for pathogenesis. *Microbiology*. 1994;140 (Pt 9):2475-9.
263. Lo Re V, 3rd, Carbonari DM, Lewis JD, Forde KA, Goldberg DS, Reddy KR, et al. Oral Azole Antifungal Medications and Risk of Acute Liver Injury, Overall and by Chronic Liver Disease Status. *The American journal of medicine*. 2016;129(3):283-91 e5.

264. Hoenigl M, Duettmann W, Raggam RB, Seeber K, Troppan K, Fruhwald S, et al. Potential factors for inadequate voriconazole plasma concentrations in intensive care unit patients and patients with hematological malignancies. *Antimicrobial agents and chemotherapy*. 2013;57(7):3262-7.
265. Cannon JP, Garey KW, Danziger LH. A prospective and retrospective analysis of the nephrotoxicity and efficacy of lipid-based amphotericin B formulations. *Pharmacotherapy*. 2001;21(9):1107-14.