Immunocompromised Patients. Infections, Diagnostics and Nosocomial Transmission

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2018

Document Version:
Publisher's PDF, also known as Version of record

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Published: 2018-03-22

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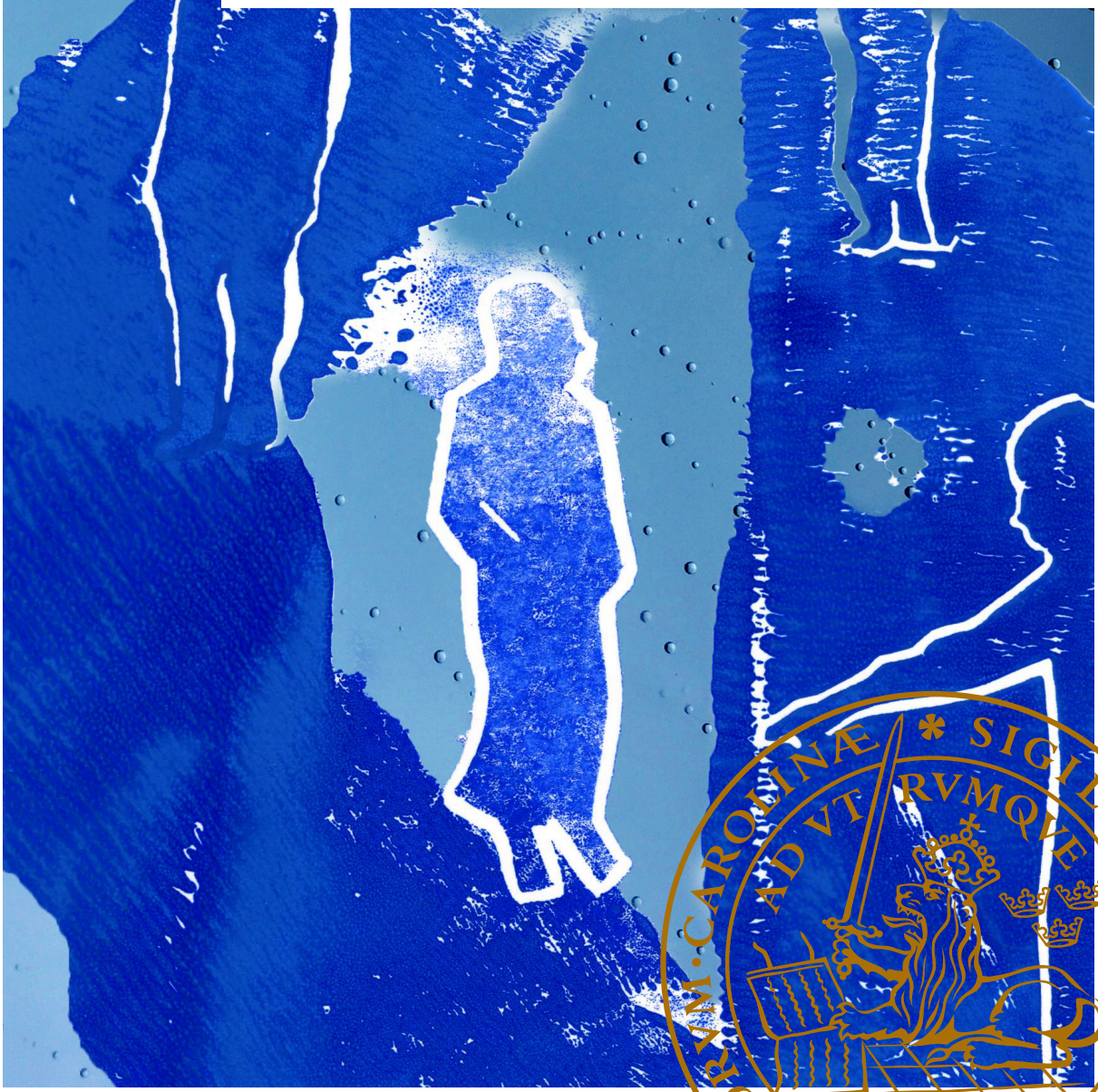

Immunocompromised Patients

Infections, Diagnostics and Nosocomial Transmission

Anna Stjärne Aspelund

LUND
UNIVERSITY

DOCTORAL DISSERTATION
which, by due permission of the Faculty of Medicine, Lund University, Sweden, will be publicly defended on 13 April 2018, at 13.00 in GK-salen, BMC, Lund University, Lund Sweden.

Faculty opponent
Britt Marie Eriksson, University of Uppsala

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION
	Date of issue
Author(s) Anna Stjärne Aspelund	Sponsoring organization

Title and subtitle: Immunocompromised Patients - Infections, Diagnostics and Nosocomial Transmission

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Haematology patients with severely impaired immunity are at high risk of developing invasive fungal disease (IFD), and early diagnosis remains a challenge to clinicians. In a study of 135 severely immunosuppressed haematology patients it was found that both (1-3)-β-D-Glucan (BG) and galactomannan showed high diagnostic performance for exclusion of disease, but poor performance in early detection of IFD when used as screening markers. BG was shown to be the most useful tool for the diagnosis of IFD later in the course of infection. For optimal diagnostic performance, the use of the BG test should include quantification of BG above the maximum limit of detection of the assay and graphical evaluation of the dynamic pattern of BG. The diagnostic usefulness of bis(methyl)gliotoxin and the D-arabinitol/L-arabinitol ratio in urine appeared to be questionable in this cohort. It was also found that elevated levels of triglycerides could be a source of false-positive BG findings. The study further highlights the importance of assessment by a qualified radiologist in the diagnosis of IFD.

Lung-transplant patients have a high risk of microbial colonisation of the lung, and lung infections. To evaluate positive microbial findings in BALF, and to implement appropriate prophylaxis and treatment of infections, it is important to know the microbial panorama. A study was carried out on all lung-transplant patients in Sweden over a two-year period, and BALF samples from 85% of the patients had microbiologic finding(s), but the frequency of multidrug-resistant bacteria was low. The microbiological findings in BALF from patients with and without lung infection were similar, providing further evidence that microbial results should be evaluated together with clinical symptoms and macroscopic appearance and in the assessment of lung infection in lung-transplant patients.

The final study was carried out during a prolonged nosocomial outbreak of a metallo-β-lactamase-producing Pseudomonas aeruginosa strain, affecting immunosuppressed patients. This was found to be associated with hospital sink drains, and a method of decontamination using acetic acid was proposed.

Key words: Immunocompromised patients, haematology patients, lung transplant, heparin-binding protein, invasive fungal disease, (1-3)-β-D-Glucan, lung infections, nosocomial transmission, acetic acid

Classification system and/or index terms (if any)

Supplementary bibliographical information Faculty of Medicine Doctoral Dissertation Series 2018:27		Language English
ISSN and key title: 1652-8220		ISBN 978-91-7619-594-9
Recipient's notes	Number of pages	Price
	Security classification	

Signature  Date 2018-03-08

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2018

Lund University
Faculty of Medicine Doctoral Dissertation Series 2018:27

ISBN 978-91-7619-594-9
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2018

Till mina pojkar

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. **Stjärne Aspelund, A.,** Hammarström, H., Inghammar, M., Larsson, H., Hansson, L., Christensson, B. and Påhlman, L. I.
Heparin-binding protein, lysozyme, and inflammatory cytokines in bronchoalveolar lavage fluid as diagnostic tools for pulmonary infection in lung transplanted patients.
American Journal of Transplantation. 2018 18(2):444-452

II. Hammarström H., **Stjärne Aspelund A.**, Christensson B., Heußel C. P., Isaksson J., Kondori N., Larsson L., Markowicz P., Richter J., Wennerås C., Friman V.
Prospective evaluation of a combination of fungal biomarkers for the diagnosis of invasive fungal disease in high-risk hematology patients
Accepted for publication in Mycoses

III. **Stjärne Aspelund A.**, Hammarström H., Inghammar M., Larsson H., Hansson L., Riise G., Friman V., Christensson B., Påhlman L. I.,
Microbiological findings in bronchoalveolar lavage fluid of lung-transplanted patients in Sweden
Submitted

IV. **Stjärne Aspelund, A**., Sjöstrom, K., Olsson Liljequist, B., Mörgelin, M., Melander, E. and Påhlman, L. I.
Acetic acid as a decontamination method for sink drains in a nosocomial outbreak of metallo-beta-lactamase-producing *Pseudomonas aeruginosa*
Journal of Hospital Infection 2016 94(1): 13-20

Abbreviations

AMP	Antimicrobial peptide
AML	Acute myeloid leukaemia
ATG	Anti-thymocyte globulin
AUC	Area under the receiver operating characteristic curve
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BG	(1-3)-β-D-Glucan
bm-gliotoxin	Bis(methyl)gliotoxin
BOS	Bronchiolitis obliterans syndrome
CMV	Cytomegalovirus
CRF	Case report form
CT	Computed tomography
DA	D-arabinitol
EBV	Epstein–Barr virus
ELISA	Enzyme-linked immunosorbent assay
EORTC	The European Organisation for Research and Treatment of Cancer
FEV1	Forced expiratory volume in 1 second
GEE	Generalized estimating equation
GM	Galactomannan
GVHD	Graft-versus-host disease
HAI	Hospital-acquired infection
HBP	Heparin-binding protein
HHV	Human herpesvirus
HSV	Herpes simplex virus
HSCT	Haematopoietic stem cell transplantation
ICU	Intensive care unit
IFD	Invasive fungal disease

IL	Interleukin
ISHLT	International Society for Heart and Lung Transplantation
IQR	Interquartile range
LA	L-arabinitol
LTx	Lung transplantation
MBC	Minimum bactericidal concentration
MBEC	Minimum biofilm eradicating concentration
MBL	Metallo-beta-lactamase
MDR	Multidrug-resistant
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MIC	Minimum inhibition concentration
Pae-MBL	MBL-producing *Pseudomonas aeruginosa*
PAD	Pathological anatomical diagnosis
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PJP	*Pneumocystis jiroveci* pneumonia
ROC	Receiver operating characteristics
TNF	Tumour necrosis factor
VZV	Varicella zoster virus

Abstract

The aim of the work presented in this thesis was to improve the management of infections in immunocompromised patients by studying aspects of diagnostics, epidemiology and nosocomial transmission. Infection and rejection are common complications in lung-transplant patients, and early diagnosis and treatment are important for a positive outcome. In a study of lung-transplant patients it was found that heparin-binding protein, IL-1β and IL-8 in bronchoalveolar lavage fluid (BALF) could be useful biomarkers for the detection of pulmonary infection. These biomarkers also seemed to discriminate between infection and rejection.

Haematology patients with severely impaired immunity are at high risk of developing invasive fungal disease (IFD), and early diagnosis remains a challenge to clinicians. In a study of 135 severely immunosuppressed haematology patients it was found that both (1-3)-β-D-Glucan (BG) and galactomannan showed high diagnostic performance for exclusion of disease, but poor performance in early detection of IFD when used as screening markers. BG was shown to be the most useful tool for the diagnosis of IFD later in the course of infection. For optimal diagnostic performance, the use of the BG test should include quantification of BG above the maximum limit of detection of the assay and graphical evaluation of the dynamic pattern of BG. The diagnostic usefulness of bis(methyl)gliotoxin and the D-arabinitol/L-arabinitol ratio in urine appeared to be questionable in this cohort. It was also found that elevated levels of triglycerides could be a source of false-positive BG findings. The study further highlights the importance of assessment by a qualified radiologist in the diagnosis of IFD.

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The final study was carried out during a prolonged nosocomial outbreak of a metallo-β-lactamase-producing *Pseudomonas aeruginosa* strain, affecting immunosuppressed patients. This was found to be associated with hospital sink drains, and a method of decontamination using acetic acid was proposed.

Introduction

The number of patients with a compromised immune system, known as immuno-compromised patients, is increasing as the result of modern therapies such as chemotherapy, stem-cell transplantation, solid-organ transplantation and the treatment of autoimmune diseases. Patients treated for haematological malignancies and lung-transplant patients are at particular risk due to the potent immunosuppression required and are the main groups of interest in the present studies.

Immunosuppression increases the risk of infections, ranging from common bacterial and viral infections circulating in the community, to opportunistic infections such as invasive fungal disease (IFD). The total risk of infections depends on several individual factors such as, the underlying disease, duration and degree of immunosupression and on which part of the immune system that is affected.

Early diagnosis and treatment of these infections are crucial for a favourable outcome. However, the classical signs of infection, e.g. fever, neutrophils and radiological findings, are often lacking in immunocompromised patients, even in the case of severe infections. Moreover, infections may be confused with rejection and graft-versus-host disease (GVHD), as for lung transplanted patients were lung infections may be difficult to clinically distinguish from rejection. Diagnosing infection in immunocompromised patients is thus challenging and there is a need of additional diagnostic tools. In particular in the case of IFD, where there are few reliable diagnostic biomarkers.

Antimicrobial therapies are often complex due to the need for early empiric treatment, drug toxicity and drug interactions. In the choice of empiric therapy, knowledge of the local epidemiology is important.

Aggressive treatment requires long hospital stays, leading to an increased risk of infections associated with the hospital environment, so-called nosocomial infections. *Pseudomonas aeruginosa* is a well-known pathogen, which can survive in moist environment and cause nococomial infections. Furthermore, multi-drug resistant (MDR) bacteria is an increasing problem, which affects in particular immunocompromised patients.

Background

Inflammation and the immune system

In this chapter, the basic principles of the normal immune system are outlined, focusing on the topics dealt with in this thesis.

The innate immune system provides a first line of defence against microorganisms. After penetrating barriers such as the skin, respiratory or gut epithelium, the innate immune system is triggered within minutes to hours and attacks invading microbes with the aim of eliminating them. The innate immune system consists of the contact system, complement, antimicrobial peptides and different kinds of blood cells. Neutrophils and macrophages are particularly important in this initial defence [1].

Antimicrobial peptides

Antimicrobial peptides (AMPs) are part of the innate immune system, and are produced in the lung and other mucosal tissues, where they act as the first line of defence against infections [2]. Different AMPs are expressed depending on cell and tissue type [3, 4], and more than 2000 naturally occurring AMPs have been identified. Most AMPs kill microbes by disrupting their cell membrane [5, 6]. Certain AMPs also recruit neutrophils and/or induce cytokine production [7]. Lysozyme is the most abundant airway AMP and is primarily secreted by neutrophils and sub-mucosal glands [8].

Neutrophils

Neutrophils are the most common kind of white blood cell in the circulation, but they are considered to have a short circulatory half-life of less than one day [9]. Neutrophils are rapidly recruited to the site of inflammation where they kill invading microorganisms by the process of phagocytosis. They also have other functions in the immune system by the release of granules, the formation of neutrophil extracellular traps and the secretion of cytokines [10, 11]. Neutrophils carry proteases and proteins in granules that participate in killing microbes [12]. There are

three forms of granules, azurophilic (primary), specific (secondary) and gelatinase (tertiary)[13].

Heparin-binding protein

Heparin-binding protein (HBP) is stored in the secretory and azurophilic granules of neutrophils [14]. HBP was initially recognised for its broad antimicrobial activity, but is now known to be a multifunctional inflammatory mediator that induces vascular leakage and acts as a chemoattractant and activator of monocytes [15-17]. The secretory granules are rapidly released by neutrophil activation and HBP facilitates neutrophil extravasation by upregulating attachment receptors on the endothelial surface [18, 19]. Larger amounts of HBP are secreted with azurophilic granule content at the site of infection [14].

Plasma HBP has been described as a promising biomarker for severe sepsis and septic shock [20]. Linder et al. showed that HBP had a better diagnostic ability for severe sepsis than several other biomarkers including the neutrophil count [21 20]. Furthermore, elevated levels of HBP have been found in cerebrospinal fluid during bacterial meningitis [22], and in urine during urinary tract infections [23].

Major histocompatibility complex

The major histocompatibility complex (MHC) is a family of molecules found on most cells of the body that recognise foreign molecules (antigens) and display them on the cell surface for recognition by T cells [24]. MHC compatibility between the donor and the recipient is important in organ transplantation as this prevents the transplanted organ from being recognised as foreign.

The adaptive immune system recognises specific antigens and is responsible for the development of an immunological memory. Antigen-presenting cells expose antigens from phagocyted microbes on their surface and lymphocytes with antigen-specific receptors attach and become activated differentiating into antigen-specific effector cells [25].

There are two main types of lymphocytes, B lymphocytes (B cells) and T lymphocytes (T cells). B cells mediate humoral immunity (i.e., they produce antibodies), while T cells mediate the cell-mediated immunity. T cells can be divided into two main categories: CD4+ and CD8+. CD4+ cells can be further divided into several subtypes defined by the function and the cytokines they produce (Th1, Th2, Th3, Th17 and Treg). Once the antigen has been removed, the antigen-specific cells undergo apoptosis. However, a small number remain and form the immunological memory, which makes the response more effective the next time the microbe is encountered [25].

Cytokines

Cytokines are signalling molecules that mediate and regulate the immune system, by acting as intercellular messenger molecules after binding to receptors on target cells. Cytokines may act on the cells that secrete them (autocrine action), on near-by cells (paracrine action), or on distant cells (endocrine action) [26]. Interleukins (ILs), a certain type of cytokines, consist of a multifunctional group that primarily mediate leucocyte communication [27].

Classical pro-inflammatory cytokines include IL-1β, IL-6, IL-8 and tumour necrosis factor (TNF). IL-1β is a potent pro-inflammatory cytokine, originally identified as an endogenous pyrogen, and is believed to induce clinical manifestations such as sleep, anorexia and fatigue [28]. IL-1β has a stimulatory effect on CD4+ T cells and is released by several types of cells such as monocytes, macrophages and epithelial cells [29, 30]. Il-6 is primarily secreted by CD4+ Th-cells, macrophages and fibroblasts, and stimulates the differentiation of activated B cells into plasma cells and IgG production. IL-6 has been suggested to be an inflammatory marker, and is considered to have hormone-like effects involved in vascular disease, lipid metabolism, insulin resistance and neuropsychological behaviour [31, 32]. Il-8 is one of the most widely studied chemokines, and is primarily secreted by macrophages. It has chemotactic and pro-inflammatory effects on neutrophils, and was initially called neutrophil chemotactic factor [33]. TNF-α is a central pro-inflammatory activator, and is primarily secreted by macrophages. TNF-α was initially recognised as being responsible for the necrosis of certain tumours [34, 35]. IL-10, in contrast, has anti-inflammatory properties that down-regulate inflammatory immune responses at multiple levels [36]. IL-10 is mainly produced by T cells, B cells and macrophages, and primarily inhibits cytokine production and mononuclear cell functions.

The first cytokine was described 60 years ago, and since then more than 300 cytokines, have been described. However, little is known about their complex interactions and functions [37]. Moreover, our knowledge concerning the intricate and complex interactions between the traditionally described innate and adaptive immune systems is constantly expanding, which will probably call into question some of our present views on the immune system.

The immunocompromised host

The introduction of modern therapies has led to an increase in the number of patients with a compromised immune system. In addition, several other conditions, such as sepsis, surgery, trauma and organ dysfunction (e.g. liver and kidney dysfunction) have negative effects on the immune system. Severe systemic inflammation results in dysfunction of both the innate and the adaptive immune system [38, 39].

Patients in intensive care units (ICUs) have a high risk of infection, due to their compromised immune system, caused by the underlying condition and the violation of normal barriers by intravenous catheters, urinary catheters, surgery and mechanical ventilation [40]. Infections can be caused by bacteria from the patient's own microbial flora (endogenous) or by microbes transferred from other patients, health care workers or the surrounding environment (exogenous) [40].

Neutropenia and neutropenic dysfunction resulting from defects in the innate immune system have been associated with an increase in the risk of bacterial infections resulting from *Enterobacteriaceae*, Gram-positive cocci, tuberculosis, and fungal infections. Defects in the cellular immune system tend to increase the risks associated with viral agents such as the herpes simplex virus (HSV), varicella zoster virus (VZV), Epstein-Barr virus (EBV), *Cytomegalovirus* (CMV), human herpesvirus, parvovirus B19 and BK virus, as well as intracellular microorganisms such as *Mycobacteria, Legionella, Listeria, Nocardia* and certain opportunistic pathogens such as *Cryptococcus, Pneumocystis jiroveci, Cryptosporidium* and *Toxoplasma*. Patients with hypogammaglobinaemia have an increased risk of infection by capsulated bacteria such as *Haemophilus influenzae, Pneumococci, Moraxella* and *Meningococci* [41-44]. However, the complex interactions between innate and cellular immunity in the defence against microorganisms makes firm generalisations difficult [45].

As outlined above, immunosuppressed patients, with varying types and degrees of immune defect, are common. However, patients treated for haematological malignancies and lung-transplant patients are at particular risk due to the extremely potent immunosuppression required. These patients are the groups of interest in the present studies.

Haematological malignancies

Haematological malignancies can be broadly classified as acute leukaemia, chronic leukaemia and myelodysplastic syndrome. Type of malignancy depends on where the mutation occurs in the normal heamtoposeis (Figure 1).

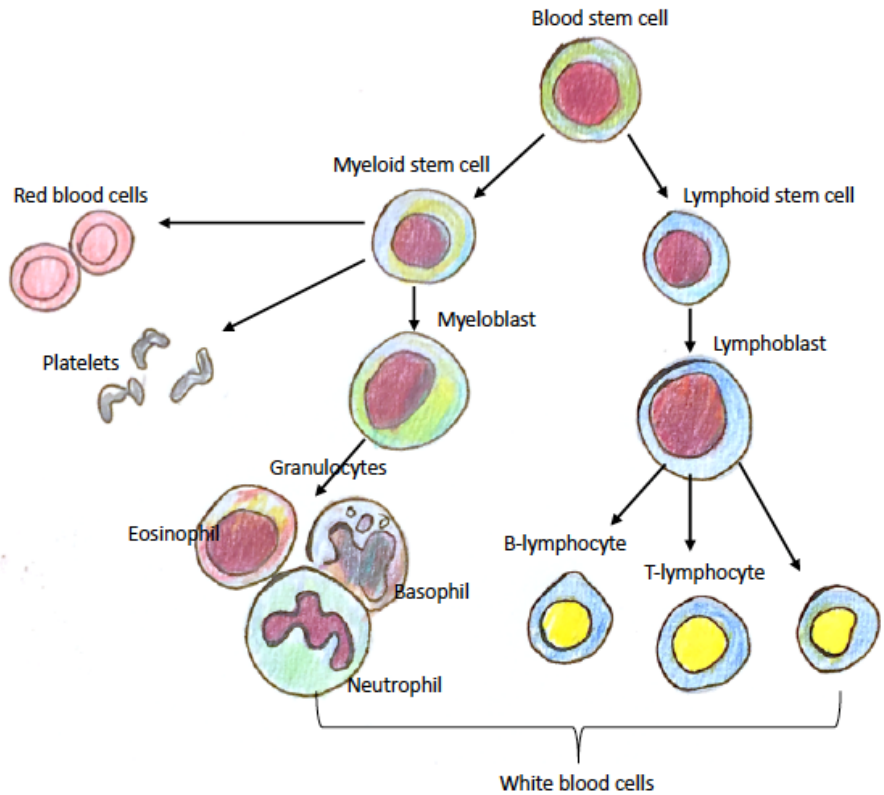


Figure 1. Normal heamtopoesis.

Acute leukaemia

Acute myeloid leukaemia (AML) is the most common type of acute leukaemia, and originates from mutations in the cells from the myeloid cell line. AML is characterised by clonal proliferation of myeloid blasts with a reduced capacity for differentiation. There are many forms of AML, and classification depends on cyto-genetic abnormalities, cell lineage and surface markers [46]. In Sweden, about 350 patients are diagnosed with AML every year, and data from the Swedish AML register indicate a five-year survival of 50-60% in patients under 50 years [47].

Acute lymphocytic leukaemia originates from mutations in cells of the lymphoid cell line [48]. This condition is most frequent in people under the age of 15 or over the age of 45, and about 150 people are diagnosed in Sweden every year [49].

Chronic leukaemia

Chronic myeloid leukaemia accounts for about 15% of all adult leukaemias. It is more common in males than in females, and mostly affects the elderly population [50]. Chronic lymphocytic leukaemia typically causes clonal proliferation and the accumulation of defect B cells in the blood, bone marrow, lymph nodes, and spleen. The condition represents approximately one-third of all leukaemia cases, affects mainly elderly patients, and has a highly variable clinical course [51].

Myelodysplastic syndromes

Myelodysplastic syndromes are a very heterogeneous group of myeloid disorders characterised by peripheral blood cytopenia and an increased risk of transformation to AML [52]. These syndromes occur more often in individuals with previous exposure to cytotoxic treatment [53].

Haematopoietic stem cell transplantation

The first successful haematopoietic stem cell transplantations (HSCTs) were performed in the early 1970s [54]. Since then, HSCT has become safer, more effective, and available for a variety of indications [55]. As for organ transplantations, MHC compatibility is important for the outcome [56, 57].

GVHD develops when the immune response of the transplanted cells is directed against the immune system, tissues and organs of the host. Acute GVHD is a well-known complication of HSCT [58, 59]. Chronic GVHD is less well understood, appears later, and resembles autoimmune disease [60]. The graft-versus-leukaemia effect is another immunological effect, which is instead favourable for the outcome. Obtaining a balance between maximizing the graft-versus-leukaemia effect and minimizing GVHD is one of the most important factors in HSCT [61]. First-line treatments of acute GVHD include high-dose corticosteroids, while second-line treatments include drugs that cause specific B and T cell depletion [59].

Treatment effect on the immune system and risk of infection

Patients with haematological malignancies are at increased risk of infection by the malignancy itself, by chemotherapy-induced immunosuppression and by cytotoxic effects on mucosal barriers [62]. The total risk of infections depends on several individual factors, such as the underlying disease, duration of neutropenia, additional cellular and/or humoral immunosuppression and the degree of disruption of normal barriers in the skin and mucosa.

Briefly, the treatment of acute leukaemia consists of induction and post-remission therapy, which includes cytotoxic chemotherapy, targeted therapies and HSCT. The induction treatment for acute leukaemia causes severe neutrophil depression, often for periods of more than 10 days, which implies a high risk of infection.

HSCT requires the almost complete eradication of the patient's own immune cells before transplantation. Immune reconstitution after transplantation takes place in several phases. The innate immunity regains its function first, while T cell reconstitution is a slower process [63]. After conditioning therapy, an "aplastic phase" follows with severe neutropenia (pre-engraftment phase) of about 14-30 days. Despite engraftment, T cells are lacking for a significant period, and reconstitution takes place after about three months [64]. Reconstitution of the B cells may take up to two years after HSCT [65, 66]. Immune reconstitution also depends on the occurrence of GVHD and the immunosuppressive treatment chosen [63].

Febrile neutropenia

Prolonged neutropenia (> 10 days with neutrophil count <100/mm^3) implies a high risk of infection [67]. Patients with severe and prolonged neutropenia often develop so-called febrile neutropenia. Empiric antibiotic therapy should be started immediately at the onset of neutropenic fever, and the treatment will remain empiric in about half of patients as it is not possible to identify a pathogen or the focus of infection [68-70]. The local epidemiology and recent history of antibiotic prophylaxis and therapy must be taken into account when choosing empiric therapy [71, 72]. In patients that do not respond to first-line antibacterial treatment, especially if duration of neutropenia more than 7 days and not on mold-active antifungal prophylaxis, empiric therapy against IFD should be started. In particular covering Aspergillus species [73].

About 50-70% of the bacteria identified in blood cultures from patients with febrile neutropenia are Gram-positive organisms, with a dominance of coagulase-negative *staphylococci,* α-haemolytic *streptococci* and *Staphylococcus aureus* [74, 75]. Among the relevant Gram-negative bacteria, *Enterobacteriaceae* and *Pseudomonas aeruginosa* are the most frequent [76]. However, Gyarmati et al. found a broad range of bacteria in blood cultures in a study of febrile neutropenic patients in Sweden using the polymerase chain reaction (PCR), which reflects the relative insensitivity of blood cultures for the diagnosis of infection in febrile neutropenia [77].

Opportunistic infections

Candida spp. and *Aspergillus* spp. are predominant fungal pathogens found in patients with haematological malignancies. *Aspergillus* is typically associated with severe and prolonged immunosuppression[78]. Invasive fungal infections are described further in a following Chapter.

Several other opportunistic pathogens, not dealt with in the present work, can cause serious infections in patients with haematological malignancies, for example CMV, *Herpes simplex,* human herpesvirus 6*,* varicella zoster virus and BK virus. In particular, CMV is a major problem in HSCT patients. The most important risk factor for the development of CMV infection in HSCT patients is the donor-positive/recipient-negative serostatus [79].

Prophylaxis

Prophylaxis against infections is generally aimed at CMV, *Pneumocystis* and fungal infections [79-82]. Fluoroquinolone prophylaxis in non-febrile high-risk neutropenic patients has been shown to reduce the risk of bacterial infections, but also raises concerns regarding increased antibiotic resistance [80, 83, 84].

Lung transplantation

The first human lung transplantation (LTx) was performed in 1963 [85]. During the following 20 years, about 45 lung transplantations were performed, all with disappointing results. The discovery of cyclosporine in the 1970s revolutionised the outcome of organ transplants, and the first successful LTx was performed in 1983 by Dr Joel Cooper in Toronto [86]. Today, lung transplantation has become an established treatment option for end-stage lung disease. The 2017 Registry of the International Society for Heart and Lung Transplantation (ISHLT) reports that a total of 60,107 adult LTxs had been performed up to June 2016 [87]. At present, about 4000 LTxs are performed worldwide each year, the number being limited by the lack of organs. The age limit for LTx has increased, and the median age of lung-transplant patients has increased from 53 years in 2004 to 58 years in 2015 [88]. In Sweden, LTxs are performed at two centres, the Sahlgrenska University Hospital in Gothenburg, and the Skåne University Hospital in Lund. About 50-60 lung transplantations are performed yearly in Sweden, the total number being about 1100 up to the end of 2017.

According to the 2017 ISHLT report, the main underlying diagnoses for adult LTx are chronic obstructive pulmonary disease (36%) and interstitial lung disease (30%), while cystic fibrosis is the underlying condition in 16% [87]. Although long-term survival has increased after LTx, the median survival is six years; one-year survival is 69%, five-year survival 59%, ten-year survival 23% and twenty-year survival only 11% [87, 88]. Lung-transplant patients in Sweden have higher survival rates, with one-year survival being 85%, five-year survival 75% and ten-year survival 60% [89].

Immunosuppression

To avoid the immune system reacting to the transplanted lung as foreign (rejection), lifelong immunosuppression is necessary. The likelihood of rejection depends on donor and recipient factors such as MHC compatibility [90]. Immunosuppression in lung-transplant patients consists of induction therapy at the time of transplantation and lifelong maintenance therapy.

The aim of induction therapy is powerful lymphocyte depletion to delay acute rejection and improve graft and patient survival [91, 92]. In Sweden, polyclonal anti-thymocyte globulin is used for induction therapy. This is a polyclonal antibody against human thymocytes (made from rabbit or horse serum), which causes significant T cell depletion [93].

Maintenance immunosuppression is also aimed at the lymphocytes, and includes a calcineurin inhibitor (cyclosporine or tacrolimus), an anti-proliferative agent (mycophenolate mofetil, sirolimus, or everolimus) and corticosteroids. According to the latest ISHLT report, the most common maintenance immunosuppression consists of tacrolimus plus mycophenolate mofetil [87]. Calcineurin inhibitors bind to intracellular structures in T cells, which inhibits activation and proliferation [94]. Calcineurin inhibitors have a poor and variable oral absorption and it is necessary to measure the concentration in blood [95]. Mycophenolate mofetil inhibits an enzyme responsible for T and B cell production [96]. The current induction therapy and maintenance immunosuppression used at the two centres in Sweden performing LTxs are outlined in Table 1.

Table 1.
Standard immunosuppression and prophylactic regimes used in Sweden

	Gothenburg	**Lund**
Immunosuppression Induction Maintenance	Thymoglobulin 2 doses, then guided by CD3 Cyclosporine/Tacrolimus MMF/Azathioprine Prednisolone	Thymoglobulin, 3 doses Cyclosporine/Tacrolimus MMF/Azathioprine Prednisolone
Per-operative antibiotics	Cefotaxime	Imipenem
Infection prophylaxis Fungal CMV Herpes PJP	 Nystatin Valganciclovir Aciklovir [2] TMS	 Fluconazole Valacyclovir/Valganciclovir [1] TMS

1 if CMV mismatch, 2 Herpes prophylaxis if CMV-/-
PJP= Pneumocystis jiroveci pneumonia MMF: Mycophenolate mofetil; TMS: Trimethoprim sulfamethoxazole

Complications

Infection and rejection are the most common acute complications after LTx, and early identification is crucial for positive outcome [97, 98].

Rejection

Almost one third of adult lung-transplant patients experience at least one episode of acute rejection requiring treatment during the first year after surgery [87]. The lung is more susceptible to injury and infection than other transplanted organs due to constant exposure to the environment through inhaled air. Immune activation in the lung caused by injury and infection is also believed to contribute to higher rates of rejection than seen in other transplanted organs [99]. Two different immune mechanisms can cause rejection; acute cellular rejection (T-cell rejection) and antibody-mediated rejection (humoral rejection).

Acute cellular rejection is the most common type of rejection, and is primarily caused by T cell recognition of foreign MHC in the lung as non-self [100]. Symptoms of rejection range from low-grade dyspnoea, reduced forced expiratory volume in 1 second (FEV1) and low-grade fever, to more acute forms of graft failure and lung dysfunction. Pulse administration of steroids is the first-line therapy. However, there are no clear guidelines for the dosage of steroids or duration of treatment, and treatment protocols vary between centres [93, 101].

ISTHL Classification and Grading of Pulmonary Allograft Rejection is outlined in Table 2 [102]. The pathologic anatomic diagnosis (PAD) grading of cellular rejection is based on perivascular and interstitial mononuclear infiltrates, and the presence of coexisting airway inflammation should be mentioned in the pathology report

Table 2.

ISTHL Classification and Grading of Pulmonary Allograft Rejection[102]
A: Acute rejection
Grade 0—none
Grade 1—minimal
Grade 2—mild
Grade 3—moderate
Grade 4—Severe
B: Airway inflammation
Grade 0—none
Grade 1R—low-grade
Grade 2R—high-grade
Grade X—ungradable
C: Chronic airway rejection—obliterative bronchiolitis
0—absent
1—present
D: Chronic vascular rejection—accelerated graft vascular sclerosis

Antibody-mediated rejection is due to circulating antibodies against the transplanted lung, and treatment is aimed at suppressing B cells. Plasmapheresis with the removal of antibodies from the circulation is usually reserved for patients with significant allograft dysfunction [99] [103].

Chronic dysfunction

Bronchiolitis obliterans syndrome (BOS), defined as a persistent and progressive obstructive decline in FEV1, was initially defined as a clinical correlate to chronic rejection [104]. Several risk factors have been identified for the development of BOS, among them infection and bacterial colonisation [105]. Chronic lung allograft dysfunction is a relatively new term that includes BOS and other conditions, and was introduced to describe any chronic decline in FEV1, irrespective of the cause [106].

Inflammatory response in the lung

Antigens are deposited on the respiratory epithelium with every breath we take. The structural integrity of the respiratory epithelium is maintained by strong connections between the epithelial cells [107]. Alveolar macrophages and interstitial macrophages in the lung play a key role in initial defence by phagocytosing invading microorganisms and secreting cytokines to recruit inflammatory cells [108]. The epithelial lining creates a further defence by secreting proteins such as mucins, cytokines and AMPs, and the disposal of antigens and microbes is enhanced by mucociliary transport [109-111].

Infections

Immunosuppression in lung-transplant patients primarily induces lymphocyte depletion and defects in the adaptive immune system. Doses of maintenance therapy are successively reduced with time after transplantation. However, if treatment is required for rejection, the patient is transferred back to a more vulnerable state with regard to infections [101].

The constant exposure to the environment through inhaled microorganisms, together with denervation, reduced ciliary transport and decreased ability to cough contributes to a high risk of microbial colonisation and lung infections. Charlson et al. found that lung-transplant patients have a higher bacterial burden in BALF than healthy control subjects, regardless of the underlying indication for transplantation [112]. However, symptoms of lung infection are often difficult to distinguish clinically from rejection, and the evaluation of a positive microbiologic finding as infection or colonisation is difficult.

Lung-transplant patients have the highest incidence of bacterial pneumonia among patients undergoing organ transplantation [41]. Clinical studies have shown that 30-70% of lung-transplant patients have a lung infection during the first year after

LTx [113-118]. The first three months after LTx are reported to be the most critical period for infections, especially those of bacterial aetiology [119, 120]. In particular, *Pseudomonas aeruginosa* is an important pathogen in lung infections in lung transplant patients [113, 116, 120],

Fungal infections are second to bacterial infections. Invasive aspergillosis is reported to occur in about 10% of lung transplant patients [121, 122]. Invasive fungal infections are dealt with in more detail in the following Chapter.

Kumar et al. found respiratory viruses in about 30% of patients during the first year after LTx [123]. Respiratory viral infections circulating in the community, such as influenza, respiratory syncytial virus, parainfluenza virus, enterovirus, rhinovirus and adenovirus, may cause severe lower-tract respiratory infections in lung-transplant patients [124].

Systemic infections caused by viruses such as CMV, HSV and VZV may cause severe infections, and lung-transplant recipients have the highest risk of developing CMV of all solid-organ-transplanted patients [125]. The most important risk factor for the development of CMV infection is the donor-positive/ recipient-negative serostatus of the transplant patient [125-127].

Definition of lung infection

There is no universally accepted definition of infection in lung-transplant patients, and definitions in the literature vary widely. The ISHLT working group on definitions of infectious diseases has proposed grading based on: 1) clinical signs and symptoms, 2) radiology, 3) microbiology and 4) histopathology [128]. The ISTLT criteria for bacterial pneumonia and colonisation are given in Table 3.

Table 3.
ISTLT criteria for bacterial pneumonia and colonisation in cardiothoracic transplant recipients

Infection	Signs/symptoms	Radiology	Microbiology/pathology	Histopathologic evidence of AR
Proven pneumonia, AR-associated OR not AR-associated	At least one of the following: ● Fever 38 °C or hypothermia 36.5 °C with no other recognized cause ● Leukopenia (4,000 WBC/mm^3) or leucocytosis (15,000 WBC/mm^3) And at least two of the following: ● New-onset of purulent sputum OR change in character/quantity of sputum OR increased respiratory secretions suctioned ● New-onset or worsening of cough, dyspnoea, tachypnoea, OR pleural rub, rales OR bronchial breath sounds ● Worsening gas exchange (O2 desaturation, PaO2/FIO2 <240, increased O2 requirements, increased ventilation demands) ● Pleural effusion	New/worsening radiographic changes on chest X-ray or CT scan	At least one of the following: ● Positive growth in blood culture unrelated to other source ● Positive growth in culture of pleural fluid ● Positive respiratory culture (sputum, bronchial secretions, BAL, bronchial protected sterile brushing samples) ● 5% BAL-obtained cells containing intracellular bacteria on direct microscopic examination	AR may be present OR absent OR not investigated
Probable pneumonia	As for proven	As for proven	Negative microbiology PLUS absence of AR by histopathology	AR must be excluded
Possible pneumonia	As for proven	As for proven	Microbiology negative or not performed PLUS concomitant clinical diagnosis of AR (without histopathology)	No histopathology performed
No pneumonia, proven AR	As for proven	As for proven/ Negative	Microbiology PLUS AR proven by histopathology	Histopathologic evidence of AR
Colonisation	Asymptomatic OR no significant changes in symptoms; stable PFT normal bronchoscopy without: ● Endobronchial erythema AND ● Purulent secretions	Absent or unchanged	Recovery of pathogen in absence of clinical or radiographic changes	AR present or absent

AR = acute rejection

Donor-associated infections

Although microorganisms are abundant in the donor lung at the time of transplantation, in particular *Staphylococcus aureus*, this does not seem to lead to post-transplant infections [129, 130]. However, a relation between donor positivity and inferior recipient outcome has been reported [131].

Perioperative antibiotic strategies vary from centre to centre. In Sweden cefotaxime or imipenem are used as routine perioperative antibiotics and are modified according to donor lung culture results once available. In lung-transplant

patients with certain lung infection problems (often CF patients) perioperative treatment is determined according to the last recorded resistance profile. Current immunosuppressive treatment and infection prophylaxis at the two centres in Sweden are outlined in Table 1.

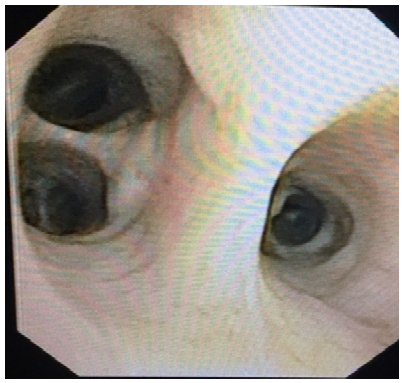
Airway colonisation and lung microbiome

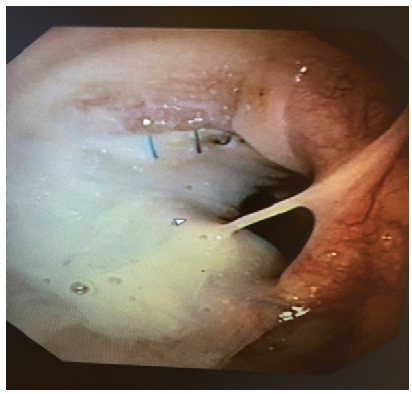
New techniques for culture-independent microbial identification have demonstrated that the lungs contain diverse communities of microbes, even in the absence of infection [132, 133]. A study on the lung microbiome in lung-transplant patients has revealed a higher bacterial burden in BALF, compared to healthy control subjects, regardless of the underlying indication for transplantation [112]. This abundance of bacteria has been suggested to be the result of increased aspiration and the inability to clear aspirated bacteria, due to impaired coughing ability and mucociliary clearance [134]. Furthermore, dominant organisms and reduced microbial diversity have been found more frequently in the respiratory tract of lung-transplant patients. It is thought that the microbial populations in the lung may trigger inflammatory pathways, resulting in graft injury; for example, colonisation by *Pseudomonas aeruginosa* has been shown to be associated with the development of BOS [135, 136].

Fibre-optic bronchoscopy and broncheoalveolar lavage

Flexible fibre-optic bronchoscopy is an effective tool for diagnosing infection and acute allograft rejection in lung-transplant patients [137, 138]. Lung-transplant patients undergo BAL several times during their first year after transplantation, both for surveillance and in response to clinical symptoms. However, the necessity of surveillance bronchoscopies has been debated [139, 140]. Lehto et al. found the diagnostic yield to be 61% in surveillance, versus 15% in indicated bronchoscopies [141]. Smith et al. retrospectively studied the results of 3734 flexible bronchoscopies, including 2111 with trans-bronchial biopsies, and reported a frequency of complications of 0.7% [142]. Standardised methods for surveillance bronchoscopies have been shown to reduce complication rates [143]. However, there is no clearly standardised method for BAL in lung-transplant patients, and procedures differ between centres.

The sensitivity of BALF for obtaining microbiological diagnosis has been reported to be between 41% and 69% [141, 144]. It has also been argued that microbiological findings in BALF may represent carryover from the oropharynx or upper airways, rather than the microbiology in the lower respiratory tract [112]. A threshold of 10^4 CFU/ml in BALF is commonly used to define pneumonia. However, opinions differ concerning the usefulness of quantitative cultures from BALF due to the inevitable dilution effect with a variable amount of fluid instilled and retrieved [145, 146].





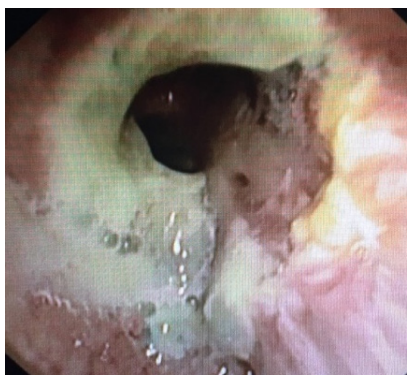


Figure 2.
Bronchoscopy macroscopic aperarance: A.Normal endobronchial mucosa, B. Purulent secretion at anastomose, C. Inflamed endobronchial mucosa with endobronchial lesion (white/yellow).
Bronchoscopy images from LTx patient, with permission from bronchocsopy unit Lund University Hospital.

Invasive fungal infections

Fungal infections occur in more than a quarter of the world's population, the majority of which are superficial. However, fungi also cause invasive diseases that are associated with high mortality, causing an estimated 1-5 million deaths per year [147]. Invasive fungal diseases (IFDs) in particular pose a serious threat to immunocompromised patients. IFDs are major causes of morbidity in patients with haematological malignancies and in HSCT patients, and those who have received solid-organ transplants [148-150]. The epidemiology of IFDs has changed over time, reflecting advances in treatment, more severely immunocompromised patients, increased control of bacterial infections and widespread use of fungal prophylaxis [151]. There are also geographical differences in the burden of IFDs, both between countries, and between regions in the same country [152].

Traditionally, the adaptive immune system has been considered more important for fungal immunity. However, it is being increasingly recognized that the adaptive and innate immune systems are intricately related [153]. The cytokine IL-17 seems to be a risk factor for invasive aspergillosis. Knowledge concerning anti-fungal immunity has advanced rapidly in the past decade, and several immunotherapeutic options, such as T cell transfusion and vaccination, are currently being evaluated [154, 155].

Invasive candida infections

Invasive candidiasis is the most common fungal disease among hospitalised patients, and presents as a deep-seated tissue infection or candidaemia. Deep-seated candidiasis is a consequence of haematogenous spread or inoculation. When candida disseminates, many organs may be involved, e.g., the kidney, liver, spleen, eye and brain. In neutropenic patients, the liver and spleen are often involved. Most epidemiological studies describe candidaemia [156]. However, the low sensitivity of blood cultures, typically around 50% depending on blood volume, culture system and ongoing fungal prophylaxis [157], may influence the reported incidence of invasive candidiasis.

In the 1980s, invasive candidiasis, dominated by *Candida albicans*, was the prominent IFD in patients treated for haematologic malignancies. Invasive candida infections have become less common in these patients as a result of the use of fungal prophylaxis [158, 159]. *Candida* non-*albicans* (e.g. *C. parapsilosis, C tropicalis, C. glabrata* and *C. krusei*) now accounts for the majority (>80%) of invasive candida infection episodes in many haematology units [160, 161]. Pagano et al. recently reported a candidaemia incidence of 1% among patients treated for haematologic malignancies in Italy [159], while Andes et al. reported an overall

invasive candidiasis rate of 8% in lung-transplant patients, 31% of which had pulmonary infections [162].

Due to the widespread use of fungal prophylaxis, invasive candidiasis is now increasingly more likely to occur in non-neutropenic patients in ICUs [156]. Well-recognized factors involved in invasive candidiasis are surgery, total parenteral nutrition, fungal colonisation, renal replacement therapy, infection, sepsis, mechanical ventilation and diabetes [163]. Recently, specific immune defects, such as defects in certain cytokine receptors, have also been described as risk factors for invasive candidiasis [164-166].

Invasive mould infections

Invasive aspergillosis is the most common invasive mould infection, and *Aspergillus fumigatus* the most frequently isolated species [167]. The main site of infection is the lung, but other organs (e.g., the brain, kidney and liver) can be involved in disseminated disease.

Steinbach et al. studied the underlying conditions of 960 patients with invasive aspergillosis and found that 48% had an underlying haematological malignancy, 28% were HSCT patients and 29% were solid-organ-transplant recipients [167]. The prevalence of invasive aspergillosis in hematology patients has been reported to be around 10%, resulting in 20-60% mortality [168-170]. Lung-transplant patients have the highest risk of invasive aspergillosis among solid-organ-transplant patients, with incidences around 10% and a mortality rate of over 20% [150, 171, 172]. In a Swedish study by Klingspoor et al., of 100 patients with invasive mould infections, 70% had an underlying haematological malignancy with a 90-day survival of 43% [173].

Non-Aspergillus mould infections

Mould in the environment can cause opportunistic infections called mucormycosis. The majority of such infections are caused by *Rhizopus* spp. followed by *Muco*r spp. The site of infection seems to vary depending on the underlying condition. The majority of patients with malignancy suffer pulmonary disease, while rhino-cerebral disease is more frequent in patients with diabetes [174, 175].

Fusarium is another environmental filamentous fungus and an opportunistic human pathogen. *Fusarium* is primarily a plant pathogen, but can cause a range of infections in humans, from superficial infections in nails and skin in the immunocompetent, to invasive disease in the immunocompromised patient [176].

These non-*Aspergillus* moulds are even more difficult to diagnose and treat than *Aspergillus*-related infections. Non-*Aspergillus* mould infections are increasing in frequency in immunocompromised patients, which may reflect a change in epidemiology due to the increasing use of mould-active prophylaxis [177].

Pneumocystis jiroveci

Pneumocystis jiroveci is a yeast-like fungus (formerly known as *Pneumocystis carinii,* classified as a protozoa). *Pneumocystis jiroveci* pneumonia (PJP) is a well-known and common opportunistic infection. The incidence of *Pneumocystis* infections in lung-transplant recipients is reported to be as high as 88% in patients not receiving prophylaxis [178]. Many immunosuppressive drugs and steroid treatment increase the risk of PJP infection [179]. Trimethoprim/sulfamethoxazole is the first-line treatment and prophylaxis [180].

Fungal prophylaxis

There is a lack of recommendations for the prevention, diagnosis and treatment of fungal infections in lung-transplant patients, which means that they are largely based on clinical experience and local epidemiology. However, it is generally considered doubtful that prophylaxis should be routinely given for *Candida* spp. in lung-transplant patients. The European Society of Clinical Microbiology and Infectious Diseases Study Group for Infections in Compromised Hosts suggests that nebulised amphotericin B should be given to all lung-transplant patients as first-line treatment or as prophylaxis when high-risk criteria, such as induction therapy with Thymoglobulin, are fulfilled [181]. Although universal *Aspergillus* prophylaxis is generally accepted, strategies vary widely from centre to centre [181-183].

In haematology and HSCT patients, fungal prophylaxis with fluconazole has been included in standard regimes worldwide. However, opinions differ concerning mould-active prophylaxis. Fungal prophylaxis with a mould-active agent has been reported to be associated with a lower incidence of IFD, but not with a significant reduction in the overall mortality of HSCT patients [81]. Moreover, the benefits of antifungal prophylaxis must be balanced against potential adverse effects and drug interactions [184]. However, mould-active prophylaxis is generally recommended during the high-risk periods of early post-transplant neutropenia and in the treatment of severe GVHD with high-dose steroids [185].

PJP prophylaxis with trimethoprim/sulfamethoxazole is very effective and highly recommended in high-risk patients [41]. Inhaled pentamidine may be an alternative in patients who cannot tolerate sulfa drugs. Lifelong prophylaxis is recommended in organ-transplant patients by many centres, and if discontinued, prophylaxis should be reinstated in cases of augmented immunosuppression [41].

Diagnostics

EORTC criteria

In 2002, the EORTC and the National Institute of Allergy and Infectious Diseases Mycoses Study Group produced criteria for the classification of potential cases of IFD according to the likelihood of underlying IFD into *possible*, *probable* or *proven,* which were revised in 2008 (Table 4 and 5) [186]. The EORTC criteria are mainly designed as a research or epidemiological tool, and the classification of IFD in most recent studies is based on these criteria.

Table 4.
EORTC Criteria for *proven* invasive fungal disease except for endemic mycoses

Analysis and specimen	Moulds	Yeasts
Microscopic analysis: 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	Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells – for example, Cryptococcus species indicated by encapsulated budding yeasts or Candida species showing pseudohyphae or true hyphae
Culture of sterile material	Recovery of a mould or "black yeast" by culturing a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine	Recovery of a yeast by culturing a sample obtained by a sterile procedure (including a freshly placed [<24 h ago] drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process
Blood	Blood culture that yields a mould (e.g., Fusarium species) in the context of a compatible infectious disease process	Blood culture that yields yeast (e.g., Cryptococcus or Candida species) or yeast-like fungi (e.g., Trichosporon species)
Serological analysis: cerebrospinal fluid	Not applicable	Cryptococcal antigen in cerebrospinal fluid indicates disseminated Cryptococcus

Table 5.
EORTC Criteria for probable invasive fungal disease except for endemic mycoses. NOTE. Probable IFD requires the presence of a host factor, a clinical criterion, and a mycological criterion. Cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are not met are considered possible IFD [186].

Host factors
Recent history of neutropenia (<0.5 × 10^9 neutrophils/l [<500 neutrophils/mm^3] for >10 days) temporally related to the onset of fungal disease
Receipt of an allogeneic stem cell transplant
Prolonged use of corticosteroids (excluding patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks
Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)
Clinical criteria
Lower respiratory tract fungal disease
The presence of 1 of the following 3 signs on CT:
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 bronchoscopic analysis
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 infection
1 of the following 2 signs:
Focal lesions on imaging
Meningeal enhancement on MRI or CT
Disseminated candidiasis
At least 1 of the following 2 entities after an episode of candidaemia within the previous 2 weeks:
Small, target-like abscesses (bull's-eye lesions) in liver or spleen
Progressive retinal exudates on ophthalmologic examination
Mycological criteria
Direct test (cytology, direct microscopy, or culture)
Mould in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:
Presence of fungal elements indicating a mould
Recovery by culture of a mould (e.g., Aspergillus, Fusarium, Zygomycetes, or Scedosporium species)
Indirect tests (detection of antigen or cell-wall constituents).
Aspergillosis
Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or cerebrospinal fluid
Invasive fungal disease other than cryptococcosis and zygomycosis
β-D-glucan detected in serum

Computed tomography

According to the updated EORTC definitions, a diagnosis of probable invasive fungal pulmonary disease is based on the prescience of at least one of three specific radiological findings on computed tomography (CT): 1) dense, well-circumscribed lesions with or without halo sign, 2) air crescent sign or 3) cavity [186]. As most of the published data on the diagnostic performance of fungal markers are based on the EORTC criteria, CT scans have a high impact on the

classification of IFDs [187-189]. High-resolution CT techniques have been reported to increase the sensitivity [188].

Biomarkers for IFD

Early diagnosis of IFD still poses a considerable clinical challenge due to the unspecific clinical picture, the low specificity of fungal isolation from non-sterile sites, and the low sensitivity of cultures for yeasts and moulds from sterile sites. There is thus a need for sensitive diagnostic tools capable of early detection. The pan-fungal cell-wall polysaccharide (1-3)-β-D-Glucan (BG) and the *Aspergillus*-specific antigen galactomannan have been widely studied and are included in the EORTC mycological criteria. However, published studies, meta-analyses and reviews on the diagnostic accuracy of BG and galactomannan show very diverse results regarding performance, cut-off levels, interpretation and usage [190-196]. Considerable efforts have been devoted to evaluating the diagnostic performance of BG and galactomannan in haematology patients at risk of IFD, but there is still no consensus on how to use these markers in clinical practice.

(1-3)-β-D-Glucan

BG is a cell wall component found in most fungi, except mucor and *Cryptococcus*, and has been used for the detection of IFDs in serum/plasma in a variety of clinical settings. The assay is based on a modification of the *Limulus* amoebocyte lysate pathway. *Limulus* amoebocyte lysate is extracted from blood cells (amoebocyte) of the Atlantic horseshoe crab *Limulus polyphemus* [197]. A sensitivity of 77% and specificity of 85% have been reported from one meta-analysis for proven/probable IFD (invasive aspergillosis and invasive candidiasis) [192]. However, results vary regarding performance, and recommended cut-off levels [193, 194, 198]. In another meta-analysis, BG was found to be superior in diagnosing *Pneumocystis* pneumonia compared to detecting invasive aspergillosis and invasive candidiasis, with a sensitivity of 95% and specificity of 86% [199]. However, the use of different commercial BG kits, with different manufacturing processes, different methodologies and positivity thresholds, has raised questions about the validity of pooled performance data [200].

False-positive results, due to external factors, such as intravenously administered antibiotics contaminated with fungal elements, have been a problem. However, changes in the manufacturing processes of antibiotics have resolved identified sources of false-positivity related to antibiotics [201-204]. Beta-glucans can also be found naturally in cellulose filters used in the manufacture of, for example, blood products for intravenous administration, and in cellulose-containing haemodialysis membranes, and may be a source of BG reactivity [205-211]. It was reported in a recent study that elevated BG levels can be detectable more than two weeks after intravenous administration of immunoglobulin [212].

Galactomannan

Galactomannan is an *Aspergillus*-specific antigen and cell-wall polysaccharide detectable in serum and other body fluids during invasive aspergillosis. The commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia Aspergillus, Bio-Rad) detects galactomannan using a rat monoclonal antibody. Levels of galactomannan correspond to the fungal load. An overall sensitivity of 78% and specificity of 81% in neutropenic patients at a 0.5 cut-off in serum has been reported in one meta-analysis [213]. A higher cut-off of 1.0 is recommended for galactomannan positivity in BALF [214, 215].

Recent studies have shown that galactomannan has a very limited value in the surveillance of asymptomatic high-risk patients on fungal prophylaxis with posaconazole or micafungin, as the results were either negative or false-positive. However, the same studies reported that galactomannan remained a useful biomarker for the diagnosis of symptomatic patients with clinical suspicion of breakthrough infection during prophylaxis [216, 217].

Gliotoxin

Gliotoxin, a metabolite produced by *Aspergillus* spp. during invasive growth [218, 219], has been detected in the sera of patients with invasive aspergillosis and proposed as a possible marker of invasive aspergillosis [220, 221]. Bis(methyl)gliotoxin (bm-gliotoxin), the inactive metabolite of gliotoxin, has been suggested to be a more stable and reliable marker for invasive aspergillosis [222]. Methods for the detection of bm-gliotoxin in serum using high-performance thin-layer chromatography with ultra-violet as well as mass spectrometric detection have been developed and proposed as a diagnostic tool for invasive aspergillosis [222].

The D-arabinitol/L-arabinitol ratio

D-arabinitol (DA), a metabolite of *Candida* spp. with the exception of *C. krusei* and *C. glabrata*, can be detected in serum and urine by gas chromatography and mass spectrometry in patients with invasive candidiasis [223-225]. A method developed to correct for endogenously produced arabinitol by calculating the ratio of DA and L-arabinitol (LA) in urine (DA/LA ratio) has been proposed as a useful marker to aid in the diagnosis of invasive candidiasis in haematological patients [226, 227].

PCR

The detection of fungal DNA by PCR methods has been studied for candidaemia and invasive aspergillosis, and is a potentially important diagnostic tool given that many fungal pathogens grow slowly or are difficult to isolate. However, the sensitivity of PCR methods may result in false-positive results due to contamin-

ation of samples from non-sterile sites, such as airways. The lack of commercially available methods and the use of different in-house PCR methods have made systematic evaluations difficult. However, some argue that PCR may now be mature enough for inclusion in the EORTC definitions [228].

Other biomarkers

Other biomarkers that have been used for the diagnosis of invasive fungal disease include *Aspergillus* antibodies [229], mannan antigen and anti-mannan antibody [230], and new methods such as the lateral flow device [231]. (For further information, the reader is referred to the respective reference.)

Treatment strategies for invasive fungal diseases

Treatment strategies for IFDs in high-risk patients have been widely debated. A pre-emptive approach is usually adopted at most centres, involving surveillance of high-risk patients. A recent meta-analysis, comparing empiric versus pre-emptive treatment strategies in patients with haematologic malignancies and neutropenic fever, indicated that pre-emptive treatment may decrease antifungal use without increasing mortality [232]. However, due to the lack of reliable biomarkers empiric treatment probably remains the most common initial strategy in clinical practice.

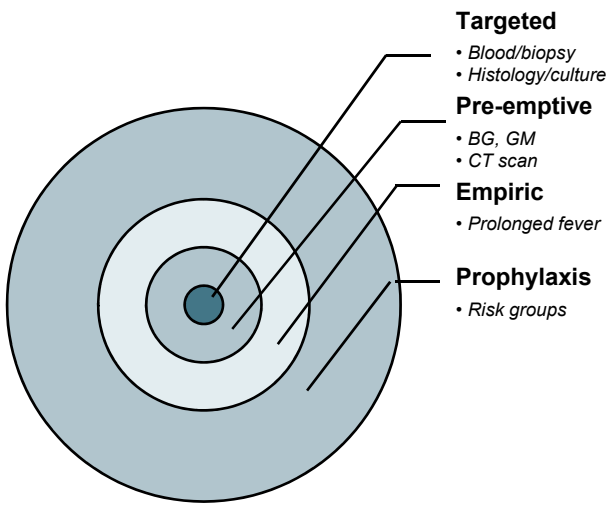


Figure 3.
Treatment strategies for IFD, from prophylactic treatment to high-risk groups. Empiric treatment implies starting treatment at prolonged antibiotic-resistant fever on clinical suspicion of IFD. Pre-emptive treatment is guided by radiological findings and biomarkers, while targeted therapy is instigated when there is clear proof of IFD [233].

Hospital-acquired infections and nosocomial transmission

Hospital-acquired infections (HAI), also called nosocomial infections, or health-care-associated infections, are defied by the WHO as [234]:

> An infection acquired in hospital by a patient who was admitted for a reason other than that infection. An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility.

Hospital-acquired infections are a major problem worldwide. The Jama network estimated that the impact on the US health care system amounted to a total annual cost of US$9.8 billion [235]. The European Centre for Disease Prevention and Control reports that 8% of patients staying in an ICU for more than two days, will present with at least one HAI [236]. Every year, approximately 65 000 patients in Sweden develop a HAI, of which around one third to one half could have been prevented. About 1500 patients in Sweden die each year as a consequence of HAIs, of which 500-750 deaths could have been avoided. The annual cost is estimated to be SEK6.5 billion, which is one tenth of the total Swedish health care budget [237].

Before the Second World War, infections occurring at hospitals were thought to originate from outside the hospital. The problem of drug-resistant bacteria connected to hospitals became obvious during the 1950s, and focus shifted to nosocomial transmission. This led to the establishment of "hospital epidemiology" departments, which in Sweden have traditionally focused on practical questions rather than epidemiology (due to the relatively low number of MDR bacteria in Sweden). The interaction between infections circulating in the community and HAIs has been acknowledged since the 1970s. For example, influenza and norovirus in the community increase the risk of the spread of influenza and norovirus in hospitals. Today, infection prevention and control teams are an integral part of most hospitals. However, the methods they employ vary widely [238].

The human microbiota is a complex ecosystem, with frequent exchange of microbes between the host and the environment [239]. Once hospitalised, this "normal" exchange of bacteria is undesirable, especially bearing in mind that the majority of patients' "ecosystems" are adversely affected by antibiotics. Contact precautions (i.e. gloves, gowns and masks) are standard when caring for patients carrying MDR bacteria. However, several recent publications recommend that

resources be focused instead on horizontal infection control strategies, which include hand hygiene, bare-below-the-elbows, chlorhexidine bathing, care bundles and environmental hygiene, to prevent the spread of MDR bacteria [240]. This is in line with "Basal Hygiene Standards", a mandatory procedure for health care personnel in Sweden [241].

Multidrug-resistant bacteria

MDR bacteria are increasing worldwide, and MDR Gram-negative bacteria are emerging as a special threat [242]. In particular, these bacteria are becoming an increasing problem in vulnerable populations [243, 244]. The WHO has declared the development of new antibiotics against carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and carbapenem-resistant and third-generation cephalosporin-resistant *Enterobacteriaceae* a critical priority [242]. The problem of MDR bacteria is less serious in Sweden and Scandinavia than in most other countries [236].

Colonisation by bacteria often precedes infection, but mapping the spread of MDR bacteria is very difficult, as many patients that have acquired MDR bacteria show negative results in initial surveillance cultures. Experience has shown, for example, that vancomycin-resistant *enterococci* may not give a positive result in surveillance cultures until several weeks after being acquired. Another problem is the identification of sites for surveillance cultures. The site of carriage and screening are rather well defined for methicillin-resistant *Staphylococcus aureus*, likewise for MDR *Enterobacteriaceae*, as these bacteria are normally present in the human flora. However, for opportunistic bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, it is more difficult to define screening sites. In the screening of 641 patients, Araoka et al. found urine cultures to be most effective for the detection of MDR *Pseudomonas aeruginosa* and recommended combining urine with throat or rectal cultures for increased accuracy [245].

Hospital environment

Maki et al. presented an important conclusion in a well-known study in 1982.

> "Our findings strongly suggest that microorganisms in the hospital environment, particularly on surfaces and in the air, but also in water, taps and sink drains, contribute to nosocomial infections occurring endemically in hospitalised patients." [246].

That microorganisms in the hospital environment contribute to nosocomial infections have been reinforced by many studies both before and after their report in;

- Studies modelling the transmission of microorganisms using computer simulations or surrogate markers [247, 248].
- Microbiological studies showing, for example, that frequently touched areas close to the patient are more contaminated [249], and that infected patients shed more pathogens than colonised [250].
- Observational epidemiologic studies, for example, that by Nosier et al., where it was found that exposure to rooms previously occupied by patients with *Acinetobacter baumannii* and *Pseudomonas aeruginosa* resulted in a higher risk of infection or colonisation [251].
- Intervention studies on cleaning and disinfection procedures, involving changing the disinfection agent or training of staff [252].
- Outbreak reports, many of which indicate that contamination of the environment plays an important role [253]. However, data are often limited by a lack of controls and the use of multiple interventions.

Hospital water systems as bacterial reservoirs

Hospital water systems and water-related devices, as well as almost any moist environment, can serve as a reservoir of pathogens in health care settings. Hospital water systems are especially prone to contamination due to their often complex structure, which leads to stagnation, corrosion, and biofilm formation [254]. Among the many sources of infection in the hospital environment, water should be considered of particular relevance due to the numerous occasions of patient exposure [255]. A wide range of hospital water reservoirs have been linked to nosocomial outbreaks [256]. Kanemori et al. recently reviewed the available literature on nosocomial transmission associated with hospital water and found that the infections included pneumonia, bloodstream infections and disseminated diseases [257]. Patient populations at risk were primarily immunocompromised patients, haematological and other malignancies, and HSCT patients, as well as patients in intensive care, neonatal and burn units. Causative pathogens included *Pseudomonas, Acinetobacter, Serratia, Stenotrophomonas, Enterobacter, Klebsiella* and *Burkholderia* [257]. Multiple hospital water-related reservoirs have been identified, such as sinks, tap water, taps, showers, baths and hospital waste-water systems [257]. The association between water reservoirs and patients thus seems clear. However, further studies are required to establish methods of preventing transmission [258].

Biofilms

Bacteria can persist on surfaces by the proctected environment created by bacterial biofilm. A common characteristic of bacteria that contaminate water systems is their ability to produce biofilms.

Biofilm formation can be described as a five-stage process: 1) initial attachment of cells to the surface, 2) production of extracellular polymeric substances (which provide the structural support for the biofilm) resulting in firmer attachment, 3) early development of biofilm architecture, 4) maturation of biofilm architecture, and 5) dispersion of single cells from the biofilm [259]. The expression of bacteria changes in a biofilm, and complex communication between organisms is established [260]. Biofilms are believed to have a considerable impact on health care, and it has been estimated that they are associated with 65% of all nosocomial infections [261]

The concentrations of biocidal agents used for cleaning in hospitals are effective on bacteria in planktonic form, but are often not sufficient to kill bacteria in biofilms. The minimum biofilm-eradicating concentration is often more than 100 times higher, and mechanical disruption of the biofilm is needed to expose the bacteria to the bactericide [262].

Sinks, sink drains and sink design

Sink drains harbour many bacteria, and sink drain locks containing antiseptics were introduced in the 1980s when bone marrow transplantations were introduced. Regular maintenance, including the replacement of sink drains and flow straighteners, was previously routine. During the past decades, less attention has been directed towards sinks and water sources, and most precautionary practices have been abandoned. However, the increase in MDR bacteria has resulted in greater attention being paid to this possible path of bacterial transmission.

It has been suggested that a sink design in which the water flows straight down from the tap onto the sink drain may enhance bacterial transmission as it causes splashing [263]. Ayliff et al. described the problem of *Pseudomonas* transmission from sink drains as early as 1971, and concluded that: "A simpler solution to the problem would be to ensure that the water from the tap is not directed directly to the sink outlet." [264].

Sinks in hospitals harbour bacteria with increased pathogenic potential due to contamination by patients that carry MDR bacteria. Contamination of the tap is thought to occur by splashing, touching or inadequate cleaning, for example, by the transfer of bacteria from the sink drain to the tap when using the same cloth for cleaning. In the investigation of an outbreak of *Elizabethkingia meningoseptica* in an ICU ward, Balm et al. found that improper use of hand basins, for example, for the disposal of patient secretions and the rinsing of re-usable patient care items, was four times more likely to have contaminated tap water compared to sinks that were not misused in this way [265].

Tap contamination has also been found to depend on the type of taps, plumbing and water flow rate. Electronic taps with complicated mechanics, and stagnant

water after the mixer valve, were found to be associated with a higher degree of contamination, especially the first portion of water delivered [266].

Interventions to prevent transmission

Various methods of preventing bacterial outbreaks associated with sink drains have been proposed, such as replacing sinks completely [267] or modifying them to prevent splashing [263]. Other measures have been implemented, including replacement with sinks that are easier to clean, reducing blockages, staff training and reviewing and improving cleaning procedures [268, 269]. Vergaz-Lopes et al. reported the removal and exchange of the whole horizontal drainage system [270, 271]. Chemical disinfection has been used, for example, sodium hypochlorite [272]. Finally, the installation of self-disinfecting sink drains has been found to be an effective solution [271, 273]. However, the methods mentioned above have not been thoroughly investigated, and more knowledge is needed.

Pseudomonas aeruginosa

P. aeruginosa is a well-known pathogen causing HAIs, especially in immuno-compromised patients. In a report issued by the European Centre for Disease Prevention and Control, *P. aeruginosa* was stated to be the most common bacterium in ICU-acquired pneumonia, the third most common in ICU-associated urinary tract infections, and the seventh most common in ICU-associated sepsis [236]. *P. aeruginosa* biofilms have also been found to play a key role in many clinical infections [274]. The ability of *P. aeruginosa* to form biofilms is considered to be one of the factors contributing to its virulence [262]. In a recent publication, Kaiser at al. described the interplay between resistance, virulence and biofilm formation in *Pseudomonas* strains found in the hospital environment. They found that *Pseudomonas* strains with many resistance genes also exhibited better biofilm-forming properties and thus had an increased ability to persist in the environment [275]. Moreover, the ability to form biofilms has been associated with higher mortality in bacteraemia caused by carbapenem-resistant *Pseudomonas* [276].

Metallo-beta-lactamase (MBL) is an enzyme produced by some bacteria, such as *P. aeruginosa,* making them resistant to beta-lactam antibiotics such as cephalosporins and carbapenems [277]. The potential for horizontal transfer of MBL makes it one of the most feared resistance mechanisms. There are different types of MBL; the most common in *P. aeruginosa* being IMP and VIM [278, 279].

Pseudomonas aeruginosa and hospital water systems

Pseudomonas can survive in moist environments and form biofilms that colonise hospital water systems [258]. Trautman et al. found that 14-50% of *P. aeruginosa* strains identified in ICU patients were also found in tap water samples [280]. Varin et al. recently showed that U-bends in high-risk units (ICU, haematology wards)

were very frequently contaminated with *P. aeruginosa* (79% of U-bends), with moderate genomic diversity [281]. Based on a systematic review, Loveday et al. concluded that water systems can act as a source of *P. aeruginosa* infection in health care settings, although the route of transmission was unclear [258]. Several nosocomial outbreaks of *P. aeruginosa* have been associated with hospital water systems, sinks, sink drains, taps and showers [263, 269, 282, 283]. In the UK, this has led to general recommendations for the management of hospital water in augmented care settings, including regular sampling and monitoring of *P. aeruginosa* in tap water [284].

Acetic acid

Acetic acid has long been used for topical wound treatment. In historical times, Hippocrates recommended the use of vinegar for the treatment of ulcers [285]. In 1916, K. Taylor reported that acetic acid was particularly effective for the eradication of *P. aeruginosa* from superficial wounds [286]. In 1968, Philips et al. reported that topical use of 5% acetic acid had a remarkable clinical effect in the treatment of wounds infected with *P. aeruginosa* [287]. Indications have also been reported that acetic acid may interfere with fibroblast activity and neutrophil function [288], however, a recent publication describing the evaluation of the cytotoxicity of acetic acid *in vitro* reported 1% acetic acid to be non-toxic to dermal fibroblasts [289]. In a developing country with multiple antibiotic-resistant strains of *P. aeruginosa*, Nagoba et al. found acetic acid to be a simple, effective and economical approach for the management of wound infections [290].

Acetic acid has also been found to be effective against other bacteria. Fraise et al. demonstrated that 0.31% acetic acid could inhibit *Staphylococcus aureus* and *Acinetobacter baumannii,* while concentrations as low as 0.17% have shown good activity against *P. aeruginosa* [291]. Furthermore, acetic acid has antibacterial activity against biofilm-producing pathogens. Bjanholt et al. showed that a mature *Pseudomonas* biofilm grown for 3 days in a continuous-flow system was eradicated by acetic acid at a concentration at 0.5% [292].

Aims

The overall aim of the work presented in this thesis was to improve the management of infections in immunocompromised patients by increasing the current knowledge concerning epidemiology, biomarkers and nosocomial transmission.

The primary aims of the studies presented in this thesis were:

I. To evaluate HBP and lysozyme in BALF as potential biomarkers for lung infection, and to determine their ability to discriminate infection from rejection in lung-transplant patients.

II. To evaluate the diagnostic performance of a combination of fungal biomarkers (BG, galactomannan, bm-gliotoxin and the DA/LA ratio) in adult patients with haematological malignancies at high risk of developing invasive fungal disease, with emphasis on describing their clinical utility for diagnosis at different times in the course of infection.

III. To examine the microbiological panorama in BALF from lung-transplant patients in Sweden during the first year post transplantation.

IV. To describe a prolonged nosocomial outbreak of a MBL-producing *P. aeruginosa* strain associated with hospital sink drains, and to evaluate a novel method of decontamination using acetic acid.

Methods

A Summary of the study design, patients and methods used in the present work is outlined in Table 6.

Patient cohorts and study designs

Paper I

This study was conducted at Skåne University Hospital in Lund. Adult patients accepted for LTx during the period from October 2012 to December 2014 were eligible for inclusion. In total, 29 lung-transplant patients were followed up for one year after transplantation. BALF samples were collected at routine scheduled bronchoscopies at 3 and 6 months after LTx, or at diagnostic bronchoscopy performed as a result of clinical symptoms. Levels of HBP, lysozyme and the cytokines interleukin (IL)-1β, IL-6, IL-8, IL-10 and TNF were analysed in 117 BALF samples. To compensate for the dilution of BALF when estimating the biomarker concentrations in the lung epithelial lining fluid, levels of urea were analysed in concomitant BALF and plasma samples. The likelihood of pulmonary infection at the time of BALF sampling was graded according to a score of 0-3 (Table 7). Rejection was defined by biopsy PAD results. The levels of HBP, lysozyme and cytokines in BALF were then correlated to the likelihood of infection and rejection.

Paper II

This study was conducted at Sahlgrenska University Hospital in Gothenburg, and Skåne University Hospital in Lund. In total, 135 adult patients with a high risk of developing IFD, i.e., patients hospitalised due to newly diagnosed acute leukaemia, myelodysplastic syndrome or high-grade malignant lymphoma intended for curative treatment, newly diagnosed aplastic anaemia, or HSCT were included between September 2011 and December 2012, and followed for 18 months. Blood samples (n=2300) were collected for the analysis of BG, galactomannan and bm-gliotoxin, and urine samples (n=1801) for the analysis of the DA/LA ratio for three months following inclusion in the study, and at all subsequent occasions of hospitalisation during the 18-month study period. Blood samples were collected twice weekly during hospitalisation and once weekly at out-patient visits. Urine samples were collected once a week. Serum samples containing >400 pg BG/ml were diluted to obtain an exact value of BG.

Table 6.

Summary of the study design, patients and methods used in the present work.

	Study design	Participants	Follow-up	Samples analysed	Methods	Laboratory methods	Statistics
Paper I	Prospective cohort study	29 lung-transplant patients	12 months	113 BALF 77 plasma samples	Grading scale lung infections Estimated BALF dilution with urea method	ELISA FACS QuantiChrom Urea Assay Kit	Chi-squared, rank sum, Kruskal-Wallis, and Mann-Whitney U-tests ROC Logistic regression GEE
Paper II	Prospective cohort multicentre study	135 patients with haematological malignancy or allo-HSCT with high-risk of IFD	18 months	2300 serum samples 1801 urine samples	CRF Assessment of EORTC criteria Specialised radiologist assessment	Glucatell® assay kit Platelia™ Aspergillus Enzyme Immunoassay HPLC MS/MS GC-MS	Chi-squared Mann-Whitney U-test Spearman's correlation coefficient
Paper III	Prospective cohort multicentre study	126 lung-transplant patients	12 months	470 BAL	CRF Grading scale lung infections	-	Chi-squared, rank sum, Kruskal-Wallis tests Logistic regression Cox regression
Paper IV	Outbreak report and intervention study	12 patients	30 months	Surveillance of 121 sinks Intervention in 10 sinks PFGE on samples from 12 patients and 7 sinks	Epidemiological investigation Patient medical records Environmental cultures Acetic acid intervention	Pulsed-field gel electrophoresis analysis MIC MBC MBEC Electron microscopy	-

CRF Case report form, ELISA Enzyme-linked Immunosorbent assay, GC-MS Gas chromatography mass spectrometry, HPLS MS/MS High-performance liquid chromatography tandem mass spectrometry, FACS Fluorescence-activated cell sorting, MIC Minimum inhibitory concentration, MBC Minimum bactericidal concentration, MBEC Minimum biofilm eradication concentration concentration, ROC Receiver-operating characteristic analyses, GEE Generalized estimating equation models

Clinical data relevant for the assessment of the EORTC criteria and possible confounding factors in the BG and galactomannan assays were recorded in a computerised CRF. IFDs were classified based on the revised 2008 EORTC criteria, and categorised as proven, probable, possible or no IFD (Table 4 and 5). All CT scans with pathologic findings according to local radiologists, and where IFD was considered a possible cause of the radiological abnormality, were reviewed by a second radiologist specialised in fungal infections. The diagnostic performance of the fungal biomarkers was evaluated based on the selection of serum samples at different points of time during the course of infection.

Paper III

This study was conducted at Skåne University Hospital in Lund and Sahlgrenska University Hospital in Gothenburg. All adult patients accepted for LTx during the periods May 2012 to December 2014 in Gothenburg, and October 2012 to December 2014 in Lund, were eligible for inclusion. In total, 126 patients were followed for one year after transplantation. BAL was performed at routine scheduled bronchoscopies and at diagnostic bronchoscopies performed in response to clinical symptoms. In total, 470 BALF samples were included and analysed as follows: bacterial and fungal cultures, PCR for *Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila, Pneumocystis jiroveci* and a panel of respiratory viruses. Bronchoscopies with positive microbiological findings in BALF were classified as "no infection" or as "signs of lung infection" (Table 7). Clinical data were retrieved retrospectively from patient records and recorded in a computerised CRF. The first year after LTx was divided into the periods: < 1 month, 1-3 months, 3-6 months, 6-9 months, and 9-12 months. Microbiological findings were grouped into Gram-negative bacteria, Gram-positive bacteria, yeast, mould and viruses. The microbiological findings were correlated to concomitant signs of infection and background factors such as time after transplantation, underlying diagnosis and type of immunosuppression.

Table 7.
Definitions and grading of lung infections in Papers I and III

Paper	Grading of infection and rejection	A. Radiology New or increasing radiographic changes on chest X-ray or CT scan	B. Bronchoscopy One or more of the following endobronchial abnormalities: Inflamed endobronchial mucosa Endobronchial lesion (white/yellow) with/without necrotic changes Purulent secretion Inflammatory cells at cytology and/or PAD	C. Clinical criteria One or more of the following conditions: New or increased cough, dyspnoea, increased sputum Fever > 38 °C Worsening gas exchange White blood count >15	D. Microbiology One or more of the following: Bacterial growth in BALF Fungal growth in BALF Positive viral PCR in BALF Positive CMV PCR in blood	E. Trans bronchial biopsies Positive histopathology for rejection
Paper I	Definite infection	A, B, C and D				No
	Probable infection	A and/or B		C or D		No
	Possible infection	No	B or D	None	B or D	No
	No infection	No	None	None	No	No
	Rejection	-	-	-	-	Yes
Paper III	"Signs of infection"	A and/or B[1]		C and D		-
	No infection	No	None	None	Yes	-

[1] A and/or B = pneumonia; B only = tracheobronchitis; B not including inflammatory cells at cytology/PAD.

Paper IV

This study was conducted at the Skåne University Hospital, Lund. Data were collected retrospectively from patients' medical records during an outbreak of MBL-producing *P. aeruginosa* detected in 2013. Samples were collected repeatedly from 121 sink drains on three wards, and cultured for the identification of Pae-MBL. The effect of decontamination of 10 sinks with acetic acid was studied over a 30-month follow-up period. Twelve patients and seven Pae-MBL strains were typed with pulsed-field gel electrophoresis (PFGE). The anti-bacterial and anti-biofilm properties of acetic acid was investigated *in vitro*, and the biofilm and the effects of acetic acid were imaged with electron microscopy

Ethics

Studies I, II and III were approved by the Regional Ethics Committees in Gothenburg and Lund. Written informed consent was obtained from all the patients participating in the studies. Study IV was approved by the Regional Ethics Committee in Lund.

Laboratory methods

Table 6 provides a summary of the laboratory methods used in the various studies. The reader is referred to the separate papers for further details.

Acetic acid antimicrobial properties

The minimum inhibitory concentration (MIC) of acetic acid was determined by serially diluting acetic acid from 24% to 0% and incubating samples for 24 h at 37 °C in TSB. MIC was defined as the minimum concentration of acetic acid preventing visible bacterial growth. The minimum bactericidal concentration (MBC) was determined by sub-culturing 10 µl samples from previous MIC dilutions on TSBG agar and incubating them overnight. MBC was defined as the concentration of acetic acid that killed >99.9% of the inoculum. The minimum biofilm eradicating concentration (MBEC) was determined using a modification of the Calgary Biofilm Device on 24-hour biofilm [293].

Acetic acid intervention

A dose of 250 ml of 24% acetic acid was poured into sink drains of colonised sinks once weekly, and allowed to remain in the drain for 30 minutes before flushing.

Calculations and statistics

Paper I

The chi-squared, rank sum, Kruskal-Wallis and Mann-Whitney U-tests were used to assess the distribution of biomarker levels between the different classifications of infection (no infection, possible, probable, and definite infection) and rejection. Receiver operating characteristics (ROC) analysis was used to assess the diagnostic power of each biomarker for infection, dichotomized into "probable/definite infection" and "no or possible infection and rejection", by comparing the areas under the ROC curves (AUC). The sensitivity, specificity, and positive and negative predictive values were calculated based on the cut-off levels identified in the ROC analyses that maximised sensitivity and specificity.

Odds ratios (ORs) were calculated for each biomarker using logistic regression for infection dichotomized into "definite and probable" and "no or possible infection and rejection". Generalized estimating equation (GEE) models were used to account for the possibility of dependency due to multiple observations from the same patient. The different biomarkers were first analysed using univariable models, and then with models adjusted for time after LTx. All statistical tests were two-sided, and 95% CIs that did not overlap 1.0, and p-values less than 0.05 were considered statistically significant.

The ratio of urea in plasma to urea in BALF was used as a coefficient for dilution to adjust biomarker levels, as described previously by Pocino et al. [294].

Paper II

Patients with proven or probable IFD were considered true-positive cases of IFD, while patients with possible IFD were considered undetermined cases, and were excluded from the evaluation of biomarker performance. The remaining patients were considered true-negative cases of IFD. In order to enable the evaluation of BG and galactomannan, and to avoid biased results, the EORTC criteria were modified such that BG and galactomannan were valid as mycological criteria only if any additional mycological criterion was fulfilled.

The diagnostic performance of the fungal biomarkers was evaluated based on the selection of serum samples at different times during the course of infection:

- at the time of diagnosis
- within two weeks following the time of diagnosis (the sample with the maximum value of the fungal marker within two weeks from time of diagnosis)

Time of diagnosis represents the time point when the patient first fulfilled EORTC criteria leading to an IFD classification. When the date of IFD classification was based on a blood culture positive for fungi, this date was considered the true time

of diagnosis since blood cultures were drawn regularly from all patients with signs of infection. When the date of IFD classification was based on a radiological criterion, the time of diagnosis was assessed by doing an evaluation of the clinical picture during a time frame of two weeks prior to the radiological exam to account for any potential delay in diagnosis.

In cases where patients were not classified as having an IFD during the study period, the serum sample with the maximum value of the fungal biomarker, among all the samples collected during the study period, was used in the analyses.

Descriptive statistics are presented as median values with interquartile ranges. Sensitivity and specificity were determined using two-by-two tables with 95% CIs. To account for the somewhat inconsecutive inclusion of patients, and the possibility of the prevalence of IFD being affected by local prophylactic regimens, the predictive values of fungal biomarkers were evaluated using Bayes' formula at a predefined IFD prevalence. An expected prevalence of 12% was used, consistent with the prevalence of IFDs reported in other studies [170, 295, 296]. The Mann-Whitney U-test was used for comparison of medians, and Fisher's exact test for the comparison of proportions. Correlation was assessed using Spearman's correlation coefficient.

Paper III

Numerical data are presented as medians and ranges. Chi-squared, rank sum, and Kruskal-Wallis tests, and analysis of variance were used to assess the distribution of background factors among different bacterial groups, lung infections and time periods. Since participants underwent varying numbers of bronchoscopies during the study, and several patients had recurrent findings of the same pathogen in BALF, the frequency of individual microbes is reported as the total number of patients with the actual finding. Grouped microbiological findings during the first year are presented as the percent of patients undergoing bronchoscopy within the specified time period. The association between background data (gender, age at LTx, type of LTx and underlying disease, type of immunosuppression, and positive or negative donor cultures) and time to first lung infection was estimated with Cox regression.

Statistical analyses were performed using STATA/SE (version 13.1 for Windows; StataCorp LP, College Station, TX, USA), Graphpad Prism 6 (GraphPad Software; La Jolla, CA, USA) and SPSS (version 20.0; SPSS, Armonk, NY, USA).

Results

Paper I

HBP, IL-1β and IL-8 as biomarkers for pulmonary infection in lung-transplant patients

Infection and rejection are common complications in lung-transplant patients and early diagnosis and treatment are important for the outcome. In this study, it was found that HBP, IL-1β and IL-8 could be useful biomarkers for the detection of pulmonary infection in lung-transplant patients. These biomarkers also seemed to discriminate between infection and rejection.

Levels of HBP, lysozyme, and the tested cytokines increased significantly with the likelihood of infection. The concentrations in samples from patients diagnosed with rejection were significantly lower than in samples from patients with definite infection, and the levels were not significantly different from the non-infected group (Figure 4). When adjusting for the dilution factor in BALF using the urea method, HBP, IL-1β and IL-8 still increased significantly with infection, whereas lysozyme, IL-6, IL-10 and TNF did not.

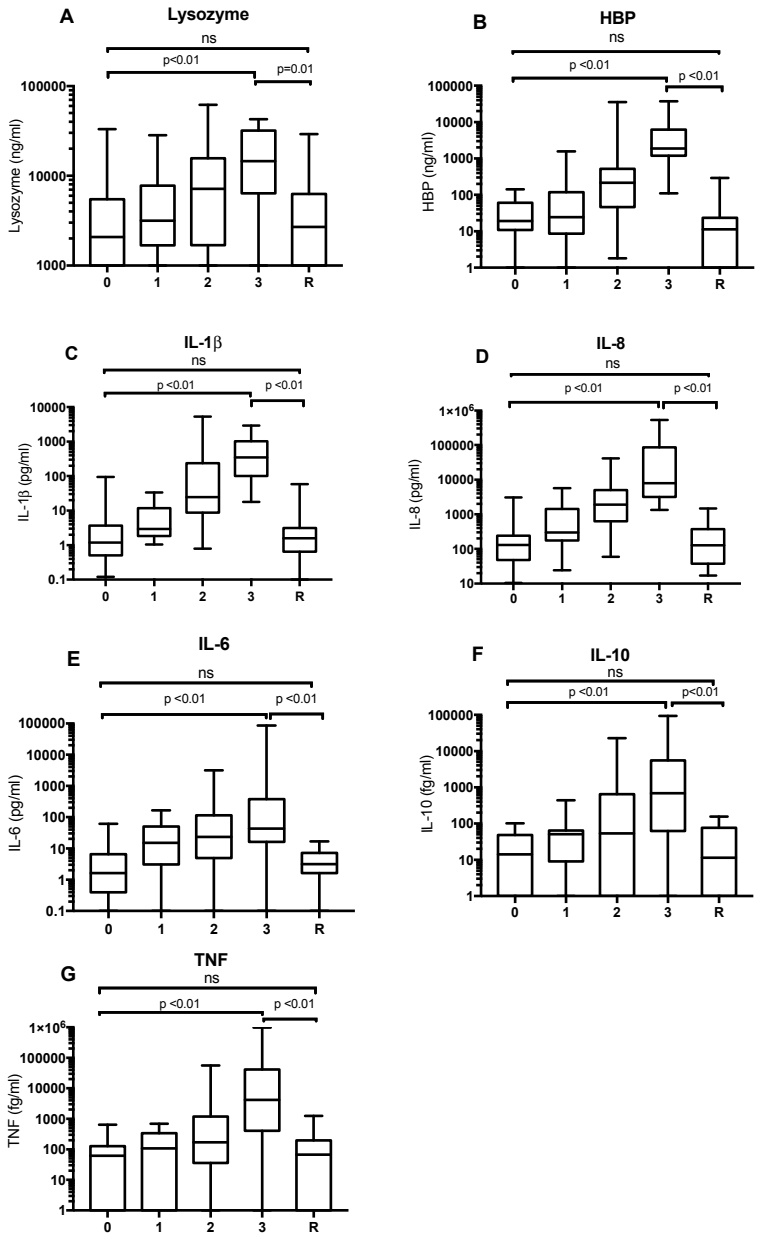


Figure 4.

Levels of lysozyme (A), HBP (B), IL-1β (C), IL-8 (D), IL-6 (E), IL-10 (F) and TNF (G) in BALF. 0=No infection (n=21), 1=possible infection (n=15), 2=probable infection (n=38), 3=definite infection(n=23) and R=rejection (n= 16). Data are presented as the median and interquartile range. Whiskers show the minimum and maximum values. Global p-values calculated with the Kruskal-Wallis test were p<0.01 for all biomarkers tested. p-values comparing separate groups (0 vs. 3, 3 vs. R and 0 vs. R) indicated by the brackets, were calculated with the Mann-Whitney U-test. ns=not significant.

At an HBP cut-off of 150 ng/ml, the sensitivity was 75% and the specificity 92% for the detection of infection; the positive predictive value and negative predictive value were 92% and 76%, respectively. The values obtained for IL-1β and IL-8 were similar to those for HBP, whereas lysozyme showed poor sensitivity and specificity (Table 8). When comparing the biomarkers to semi-quantitative analysis of inflammation in transbronchial biopsies and cytology, HBP, IL-1β, and IL-8, but not lysozyme, were significantly more sensitive in diagnosing infection than degree of inflammation (AUCs: 0.85, 0.90, 0.87, 0.67, and 0.71, respectively).

Table 8.
Sensitivity, specificity and predictive values of HBP, lysozyme, IL-1β and IL-8 in BALF for pulmonary infection in lung-transplant patients Data were calculated using two-by-two tables, where infection was dichotomized into "definite and probable infection" (n= 61) versus "no or possible infection" (n=36). The odds ratio for the prediction of definite and probable infection, compared to no or possible infection and rejection, were estimated with GEE models.

	Sensitivity (%)	Specificity (%)	PPV	NPV	Odds ratio infection
HBP (cut-off 150 ng/ml)	75	92	92	76	32
Lysozyme (cut-off 6500 ng/ml)	64	77	76	65	4
IL-1β (cut-off 10 pg/ml)	80	87	87	79	17
IL-8 (cut-off 1 ng/ml)	82	83	85	80	17

PPV = positive predictive value, NPV = negative predictive value.

Paper II

Biomarkers of invasive fungal disease

Haematology patients with severely impaired immunity are at high risk of developing IFD and early diagnosis remains a challenge to clinicians. The results of this study show that BG was the most suitable marker for the diagnosis of IFD when sampling was performed later in the course of infection, especially when quantified above 400 pg/ml together with evaluation of BG dynamics. BG and galactomannan may serve as useful tools for the exclusion of IFD and invasive aspergillosis when used as screening markers in high-risk haematology patients. However, the results do not support the use of BG or galactomannan as surveillance markers for the early detection of IFD in this cohort of patients. Elevated triglycerides were also found to be a possible source of false-positive BG results. The diagnostic role of bm-gliotoxin and the DA/LA ratio in urine in this cohort of patients seems questionable. The study further highlights the importance of assessment by a qualified radiologist in the diagnosis of IFD.

Among the 135 included patients (73% acute leukaemia, 53% HSCT) a total of 23 patients (17%) were classified as having IFDs according to the EORTC criteria [186], 13 (10%) of which were classified as having proven or probable IFD (7 cases of invasive aspergillosis, 4 cases of PJP, one case of invasive candidiasis, and one case of invasive *Fusarium* infection). Of the remaining cases, 10 were classified as having a possible IFD and 112 patients with no signs of IFD.

Both BG and galactomannan had a low sensitivity early during the course of infection (at time of diagnosis), even at the lowest cut-off levels (69% and 33% respectively). The sensitivity of BG was considerably higher (92%) when sampling was performed later during the course of infection. For galactomannan, the sensitivity was low (57%) also later during the course of infection. The specificity of BG at a cut-off level of 80 pg/ml was only 41% when using one positive sample but 80% when using two consecutive positive samples to define a positive test result. At a cut-off level of 800 pg/ml, the specificity was 100%. The specificity of galactomannan was >90% at cut-off levels 0.5 and 1.0. Combining BG and galactomannan (i.e. with the requisite of both being positive for a positive test result) at any cut-off level increased specificity but gave a very low sensitivity.

Beta-glucan dynamics

Forty-eight serum samples, from 21 patients, with a BG value of >400 pg/ml in the primary analysis, were titrated and reanalyzed to obtain an exact BG value. The results obtained from the diluted BG samples and IFD classification are shown in Figure 5. The median BG-level in patients with proven and probable IFD was significantly higher than in patients without IFD ($p<0.0001$). Eighty percent of the titrated samples from patients with IFD were higher than 800 pg/ml, whereas none of the samples from patients without IFD exceded 800 pg/ml.

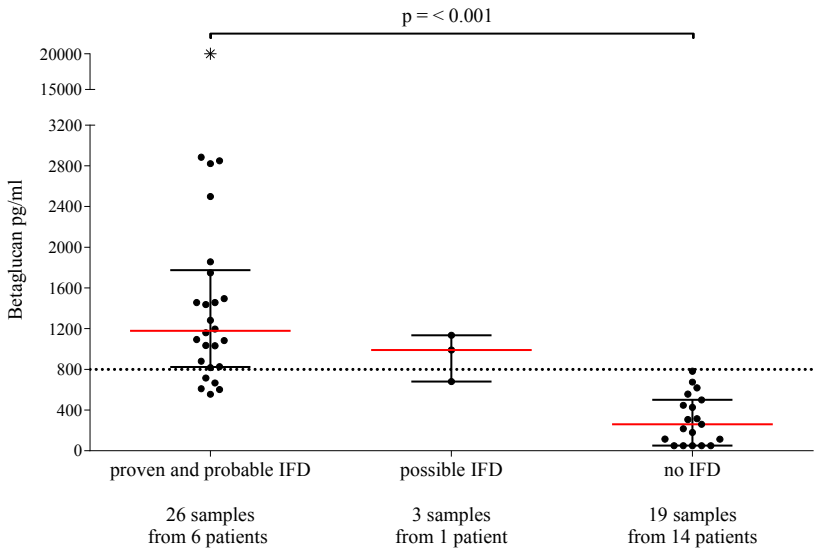


Figure 5.
Dilution of samples with beta-glucan >400 pg/ml.The horizontal red lines indicate the median values, and the error bars the interquartile range. The beta-glucan sample shown by an asterisk yielded a value of >20 000 pg/ml after dilution and reanalysis, and was obtained from a patient with proven therapy-resistant invasive aspergillosis. The groups proven/probable IFD and no IFD were compared using the Mann-Whitney test.

To determine the dynamics of BG and its relation to the presence of IFD, BG serum levels in individual patients were evaluated graphically. Samples were considered sequential if collected with a maximum interval of 7 days. For the patients with IFD, the serum samples drawn within two weeks before and after the time of diagnosis, were included in this evaluation. Seven patients without IFD and one patient with probable PJP did not have sequential BG samples and were excluded from this analysis. As shown in figure 6, four types of patterns were identified: A) only negative samples; B) isolated positive BG samples, i.e. positive samples with negative precedent and subsequent samples; C) consecutively positive samples with fluctuating levels; and D) consecutively positive samples with steadily increasing levels.

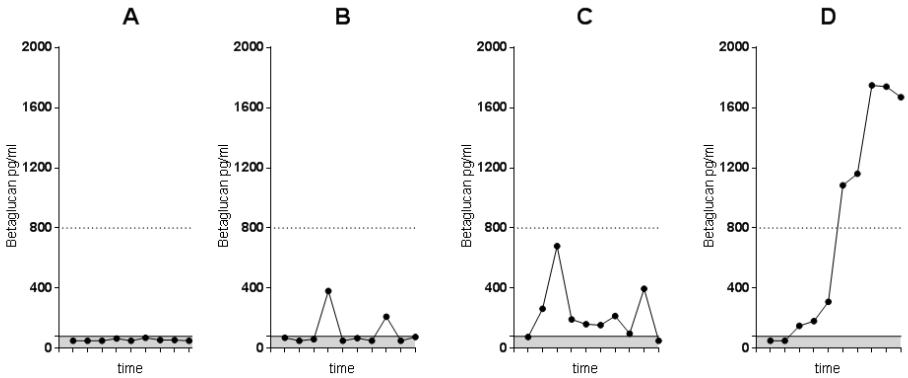


Figure 6.
Beta-glucan dynamics. The figures show representative results for the clarification of the definitions of the dynamics of BG. A) Negative samples only; B) isolated positive BG samples, i.e. positive samples with negative precedent and subsequent samples; C) consecutively positive samples with fluctuating levels; and D) consecutively positive samples with steadily increasing levels.

The clear majority of the patients with proven or probable IFD (91%) had consecutively positive and steadily increasing BG levels (pattern D). However, one patient with a probable *Aspergillus ustus* infection had only negative BG samples (pattern A), and one patient with proven *Fusarium* infection had consecutively positive but fluctuating BG levels (pattern C). Eighty-three percent of patients without IFD had either all negative or single positive BG values (patterns A or B), with no values >400 pg/ml. The remaining 17% of the patients without IFD had consecutively positive BG values (patterns C or D); however, none of these reached levels >800 pg/ml, and 27% of these patients had known triglyceridaemia.

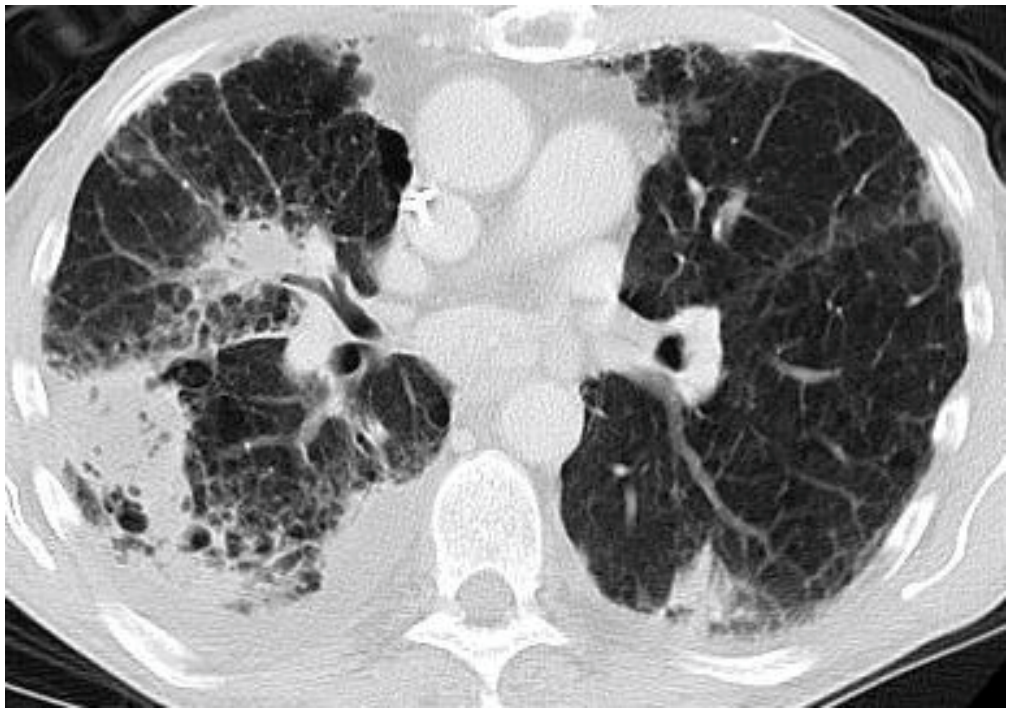


Figure 7. Local radiologist:
"Widespread patchy consolidations bilaterally, infectious origin must be suspected."
Specialised radiologist: "15 halo-signs."

The impact of radiology assessment on fulfilment of the EORTC criteria

CT scans from 32 patients with pathologic findings according to the local radiologist were additionally reviewed by a chest radiologist specialised in fungal infections, and this assessment was used for the classification of IFD in this study. We also wanted to determine whether using the assessment of a less specialised radiologist would have led to a different classification of IFD according to the EORTC criteria. The specialised radiologist found typical halo signs indicating IFD in 16 patients while local radiologists mentioned halo signs in only two patients. The term *consolidation* (meaning a well-circumscribed lesion, including macronodules) was mentioned in the local radiology reports of 19 patients, while the specialised radiologist used the same term in only 7 patients. The assessment of IFD according to the EORTC criteria based on the reports from local and specialised radiologists had different outcomes for 7 of the 32 patients (22%).

Paper III

The microbial panorama in BALF of lung-transplant patients in Sweden

Lung-transplant patients have a high risk of microbial colonisation of the lung, and thus lung infections. In order to be able to evaluate positive microbial findings in BALF, and to manage the prophylaxis and treatment of infections, it is important to know the microbial panorama. In this study, positive microbiologic finding(s) were found in samples from 85% of patients, often several pathogens. The overall frequency of MDR bacteria was low, and patterns of Gram-negative bacteria, Gram-positive bacteria, yeast, mould and viruses could be identified. The microbiological agents found in BALF from patients with lung infection were similar to the findings regarding no infection, confirming that microbial findings must be evaluated together with macroscopic appearance and clinical symptoms in bronchoscopic assessment of lung infections in lung-transplant patients.

A total of 126 patients who underwent 470 bronchoscopies were included in this study. Sixty-two percent of the BALF samples showed positive microbiological finding(s). Forty-six percent showed bacterial growth, 29% fungal growth, and the results of viral PCR analyses were positive in 9%. Only a single microbe was found in 38% of the BALF samples, while a combination of bacteria, fungi or viruses was found in 24%.

The most common microorganisms in BALF were *Pseudomonas aeruginosa, Candida albicans* and coagulase-negative *Staphylococcus* (in 41 (33%), 42 (33%) and 25 (20%) patients, respectively). Patterns of grouped bacterial, fungal and viral findings during the first year after LTx are presented in Figure 8 as the percent of patients with a bronchoscopy performed within each time interval.

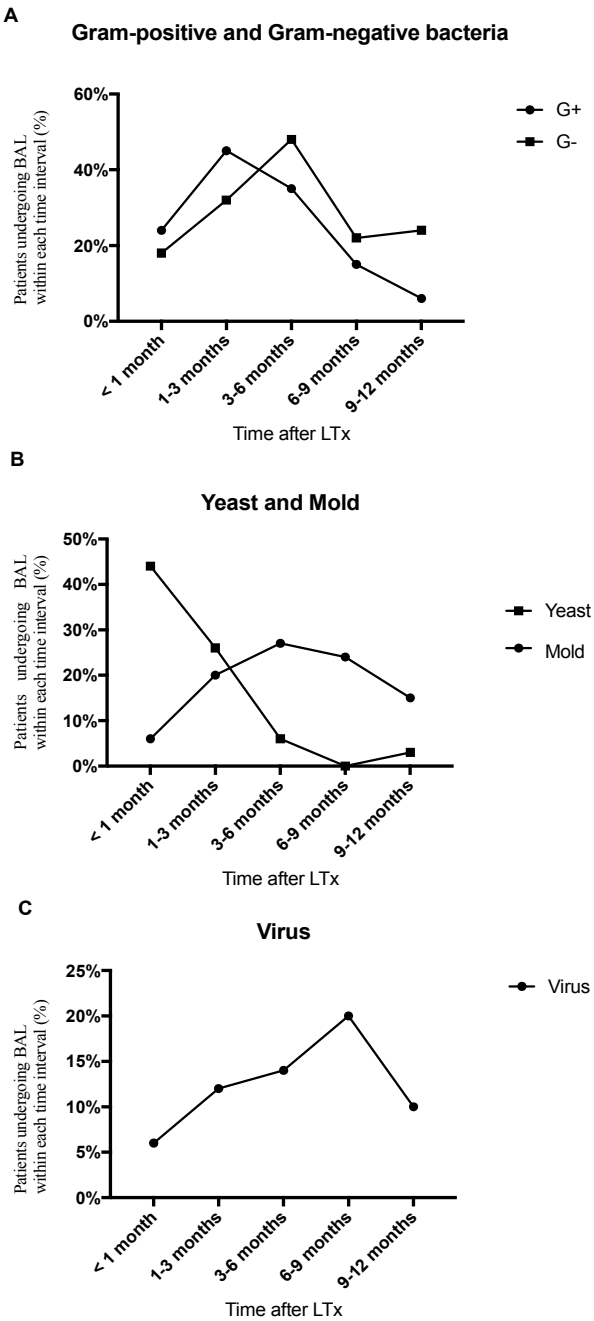


Figure 8.
Microbiological findings in BALF expressed as the percent of patients undergoing bronchoscopy within each time interval.

In 113 bronchoscopies with positive microbiological findings in BALF, the patients had clinical signs of lung infection (pneumonia or tracheobronchitis) at the time of bronchoscopy. The most common microbes in BALF from patients with signs of lung infection were *Pseudomonas aeruginosa*, *Candida albicans* and coagulase-negative *Staphylococcus* (in 24 (21%), 17 (15%) and 15 (13%) samples, respectively). The incidence of lung infections decreased over time during the first year after transplantation (Figure 9). No significant association was found between background factors (i.e. gender, age at LTx, type of LTx and underlying disease, type of immunosuppression, positive or negative donor cultures) and time to first lung infection.

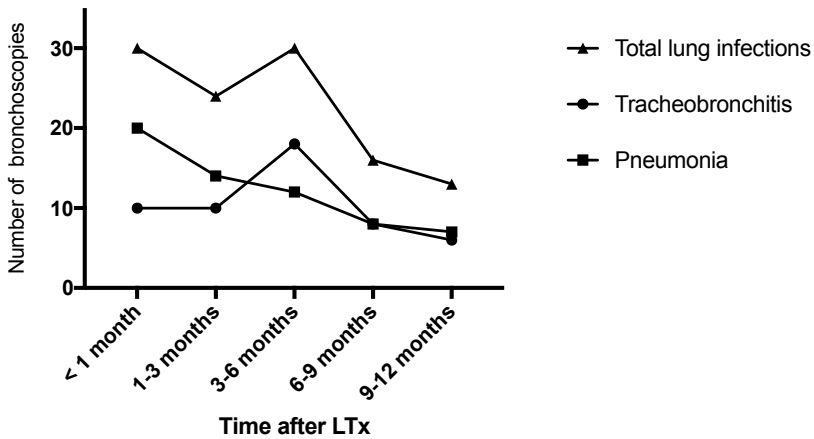


Figure 9. Number of bronchoscopies with signs of lung infection during the first year after LTx.

When comparing microbiological findings with and without signs of infection, the frequencies of Gram-negative bacteria, Gram-positive bacteria, mould and viruses were not significantly different (Table 9). Yeast was significantly more frequent with signs of infection. However, *Candida* was found as a single microbe in only 15% of samples, all with concomitant signs of tracheobronchitis, and 82% of bronchoscopies were performed within 1 month after LTx.

Table 9.
Samples with no infection vs. signs of lung infection within defined microbiological groups. The values exceed 100% as several agents were found in some samples of BALF.

Group of agents	No infection, n (%) total=159	Infection, n (%) total=113	p-value
Gram-positive bacteria	70 (44)	41 (36)	0.20
Gram-negative bacteria	64 (40)	48 (43)	0.71
Yeast	30 (19)	36 (32)	0.01
Mould	43 (27)	28 (25)	0.41
Viruses	22 (14)	18 (16)	0.48

Paper IV

Nosocomial outbreak associated with sink drains and acetic acid intervention

Two neutropenic haematology patients, who had occupied the same room, one after the other, died as a result of Pae-MBL sepsis. All patients that had been admitted to the ward during the same time period were screened for Pae-MBL. Cultures were retrieved from intravenous lines, wounds, stomas, sputum and urinary catheters but were all Pae-MBL-negative. Environmental screening revealed that the bacterium was present in the sink drain of the patient's bathroom. A search in the clinical microbiology database identified another twelve patients with positive Pae-MBL cultures between 2008 and 2014 (Table 10). Ten of the patients (83%) were deceased, and in six of these cases Pae-MBL could have contributed to mortality. The patients appeared as single cases, with a peak of four patients in the summer of 2013.

All patients except one had been treated on wards 1, 2 or 3. Three patients had been admitted to both wards 1 and 3, indicating a possible route of transmission. Wards 1 and 2 frequently exchanged patients, and Pae-MBL could have been transmitted between the wards by an undetected patient.

Table 10.
Clinical data for patients 1-12

Pat. no.	Comorbidities	First positive culture/ site of detection	Ward 1	Ward 2	Ward 3	Occupied a room with Pae-MBL in the sink drain
1	Chronic ulcer	Aug. 2008, ulcer	x			NA
2	COPD	Aug. 2008, sputum Sept. 2009, blood	x			yes
3	Ventilator associated pneumonia	Jan. 2009, tracheal secretion				NA
4	COPD	Dec. 2009, sputum	x			NA
5	UTI, pneumonia	Aug. 2011, urine	x			NA
6	Haematological malignancy, neutropenia	May 2012, blood		x		NA
7	Myastenia, Pulmonary secretion stagnation	Sept. 2012, urine Oct. 2012, sputum	x		x	yes
8	COPD	Apr. 2013, sputum	x			yes
9	Haematological malignancy, neutropenia	July 2013, blood		x		yes
10	Haematological malignancy, neutropenia	July 2013, blood		x		yes
11	Necrotizing fasciitis	July 2013, wound	x		x	yes
12	COPD	Aug. 2013, sputum	x		x	yes

COPD, chronic obstructive pulmonary disease; UTI, urinary tract infection

Cultures were obtained repeatedly from all sinks in wards 1, 2 and 3 (n=121), and a total of twelve Pae-MBL-positive sink drains were identified. All of them were in the patient bathrooms. PFGE typing of the isolates from the twelve patients and seven isolates from sinks showed identical or closely related band patterns. Information on the rooms they had occupied was available for seven patients, and all had occupied a room with a colonised sink (Table 10).

In vitro, Pae-MBL was highly sensitive to acetic acid, in both the planktonic phase and in a biofilm. The median MIC was 0.047% (range 0.047-0.094), the median MBC 0.19% (range 0.094-0.19), while the median minimal biofilm eradicating concentration (MBEC) was found to be as low as 0.75% (range 0.19-1.5).

The initial response was to replace the sinks and plumbing. However, the bacterium re-appeared in three sinks after a mean period of 13 weeks. Therefore, the traditional remedy of acetic acid was tried. A dose of 250 ml of 24% acetic acid was poured into the sink drains of colonised sinks once a week and allowed to remain for 30 minutes before flushing. Control samples were collected for culture at least every four weeks, immediately before the next treatment. In total, ten sinks showing positive results for Pae-MBL were subjected to acetic acid treatment and monitored for a median period of 38 weeks (range 24-54 weeks). Two sinks showed a positive culture after the first week of treatment, but these were negative at the second control. The smell of acetic acid could be a potential limitation but no comments or objections have been made from patients or hospital staff. Details concerning the acetic acid intervention are given in Table 11.

In July 2015, a woman with haematologic malignancy and complicated pneumonia appeared positive for Pae-MBL in sputum (pat 13). Prior to diagnosis, the patient had been admitted to ward 2 in a room with repeatedly positive Pae-MBL cultures from the wall. During her stay at the ward, the acetic acid routine had been omitted during four weeks due to staff changes and lack of communication. The fact that no further patients have been identified and that colonized sinks became culture-negative during acetic acid treatment suggests that acetic acid was important for terminating the outbreak.

Table 11.
Interventions using acetic acid, and follow-up cultures from wards 1,2 and 3

		2013						2014												2015											2016
Ward	**Room**	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	jul	Aug	Sept	Okt	nov	dec	jan
Ward 1				★★							★					★					✪	🔻			✪				✪			✪
	1			X X	✔	✔✔	✔	✔✔		✔	✔	change sink				✔☑					✔				✔				✔			✔
	4			X X	X	✔✔	✔	✔✔		✔	✔	change sink				X ☑	✔	✔	✔■ HW, CP	✔✔☑	✔✔☑				✔				✔			✔
	6			X X	✔	✔✔	✔	✔✔		✔	✔	change sink				✔☑					X				✔				✔			✔
	24			X ✔	✔	✔✔	✔	✔✔		✔	✔	change sink				✔☑					✔				✔				✔			✔
	20										X	change sink				✔■	✔	✔	✔■HW, CP	✔✔■	✔✔■				✔				✔			✔
	19											change sink				X ☑	X	✔	✔☑HW, CP	✔✔☑	✔✔☑				✔				✔			✔
Ward 2		★		★		★		★			★		★		✪	★	✪	✪	★	✪	✪	★	✪	★			★		★	✪	✪	★
	1	X change sink ✔✔	✔			✔	ward moved																🔻									
	12										X CP ✔✔		✔		✔	✔	✔☑	✔	✔	✔	✔	✔	✔	✔	*		✔		✔	✔	✔	✔
	15												X CP✔✔		X CP ✔	✔■	✔■	✔	✔	✔	✔	✔	✔	✔	*	**	✔		✔	✔	✔	✔
	9														X CP ✔	✔■	✔☑	✔	✔	✔	✔	✔	✔	✔	*		✔		✔	✔	✔	✔
	3																					X CP	✔✔	✔	*		✔		✔	✔	✔	✔
Ward 3												★																				
	10						X change sink ✔✔					✔																					

★ culture of every sink in the entire ward
✪ culture of all patient bathroom sinks
acetic acid once a week
HW hot water flushing
CP change part, sink drain and drain pipe to the wall
🔻 all patient bathroom sinks treated with acetic acid
✔ negative Pae-MBL culture from sink drain
X positive Pae-MBL culture from sink drain
☑ negative Pae-MBL culture from drain pipe in the wall
■ positive Pae-MBL culture from drain pipe in the wall
* missed acetic acid 4 weeks
** patient 13 Pae-MBL positive

Discussion

Infections

Evaluation of microbiological cultures

The evaluation of microbial findings in BALF from lung-transplant patients poses a clinical problem, which was addressed in Papers I and III.

Several publications report the causative agents in lung infections in lung-transplant patients [116, 120]. However, few state the number of samples containing poly-microbial flora. In the present studies (Papers I and III), almost half of the positive BALF samples had poly-microbial findings. In clinical practice the pathogenic potential of the identified microorganisms is often evaluated. However, it is very difficult to relate the infection to one specific finding, especially in immunocompromised patients. Several microorganisms that would normally be considered harmless were found as single microbial finding in samples with concomitant signs of lung infection. New methods of molecular analysis have led to the suggestion that the lung may harbour its own microbiome, and it is possible that the pathogenicity of individual bacteria should be reconsidered [132].

Recently published findings suggest that poly-microbial infections and interactions between pathogens play an important role in the development of pulmonary infection and inflammatory response [297, 298]. Dickson et al. reported a loss of microbial diversity and the presence of a dominant pathogen during pneumonia [132]. Shanker et al. found that samples from patients with pneumonia exhibited significantly lower microbial diversity than samples showing colonisation, whereas samples from patients with tracheobronchitis were characterised by high microbial diversity that differed from that in samples from patients with pneumonia and colonisation [299]. In Paper III no difference was found in the number of poly-microbial findings when comparing samples from patients showing signs of infection and no infection, neither when comparing pneumonia and tracheobronchitis using routine cultures. However, differences might have been seen if mapping the full microbiome.

Quantitative determinations from cultures of BALF have frequently been used to identify causative agents in lung infections. However, a variable amount of fluid is instilled during bronchoscopy and a variable volume is retrieved, and it will therefore be difficult to interpret quantitative measurements. Geberaux et al. reported

low repeatability in quantitative BALF cultures in 44 mechanically ventilated patients with suspected nosocomial pneumonia. In their study two BALs were performed on the same lung, with a thirty-minute interval, by the same operator. The qualitative repeatability (presence or absence of bacteria) was 95%, but the quantitative repeatability for bacteria was only 27% (same value of log10 for both BALs of the same patient) [300]. In the present work, the concentration of urea in concomitant serum and BALF samples was used to adjust for dilution, in a similar way to the proposed use of the urea coefficient to calculate the effect of dilution on CFU counts in BALF [146, 301].

Differences in sampling techniques may influence the microbiological results. Bello et al. evaluated the impact of different bronchoscopy sampling sites in a prospective single-centre study of 79 ICU patients. BALF cultures from the right and left lungs resulted in discordant results in approximately 40% of cases when a CFU threshold of $\log10^4$ was applied for positivity [302]. In the present study (Paper I) the majority of the BALF samples (76%) were retrieved from the middle lobe. However, no differences were found in the distribution of the biomarkers tested or urea in BALF retrieved from the middle lobe versus BALF retrieved from other locations.

Patterns of microbial findings

Of the patients studied in this work, 45% of bronchoscopied patients had yeast in BALF less than one month after LTx (Paper III). *Candida* has been reported in 40-50% of BALF samples from critically ill and mechanically ventilated patients [303]. Delisle et al. found an association between *Candida* colonisation, mortality and increased hospital stay in patients with ventilator-associated pneumonia, which may reflect a more severe underlying disease [304]. However, animal models indicate that *Candida* may increase the pathogenic potential of bacteria in the lung [305]. In particular, there seems to be a relationship between *Candida* and *Pseudomonas* [306], which is interesting as *Candida* and *Pseudomonas* were found to be dominating agents in the present study, in BALF samples both with and without signs of infection (Paper III).

The low frequency of bacterial findings during the first month after LTx (Paper III) may be the result of broad antimicrobial postoperative treatment. However, bacterial findings increased with time. Most Gram-positive bacteria were found in BALF 1-3 months after LTx, while Gram-negative bacteria peaked at 3-6 months after LTx. In a shorter perspective Durin et al. recently surveyed all microbial findings in over 5000 patients with burn injuries during their initial hospital stay, and found that Gram-positive organisms occurred earlier than Gram-negative ones, and that MDR strains of the same bacteria occurred later than susceptible strains [307]. This could mirror an initial increased risk of endogenous infections and a and later occurance of exogenous infections.

The later occurrence of mould described in Paper III was also seen in the study described in Paper II**,** where six of seven patients developed invasive aspergillosis more than 40 days after HSCT. All were HSCT patients in the post-engraftment period, showing reactivation of CMV or GVHD.

Respiratory viruses are common in the community, and this is probably reflected in the later occurrence in BALF from lung-transplant patients (Paper III). However, no seasonal variation in viral occurrence was found, and seasonal viruses such as influenza and parainfluenza were detected in only a minority of patients. Instead, coronavirus and rhinovirus dominated. Ambrosini et al. also reported rhinovirus to be very common in lung-transplant patients with a high viral load, even in asymptomatic patients [308].

Classification of infection

Different classifications of infection were used in the first three studies (Papers I, II and III), which have important implications on the results. In the first study, *definite* and *probable* infection were considered to be true infections, while *possible*, *no infection* and *rejection* were considered to indicate no infection. A probable infection was regarded as a true infection in an attempt to reflect clinical practice, as a probable infection in this setting, with very immunocompromised patients, would probably be treated in clinical practice. The primary aim of the study described in Paper III, however, was to map the microbiological panorama in BALF, and only samples with positive microbiology were considered in the classification signs of infection. However, if the grading of infection used in Study I had been applied in Study III, all cases with signs of infection would have been defined as definite infection (pneumonia) or probable infection (tracheobronchitis).

CT of the thorax is a very important tool in diagnosing invasive aspergillosis, especially as bronchoscopy in haematology patients is sometimes not advisable due to low platelet counts nd the risk of complications. In today's clinical setting, radiology generates more information than biomarkers in the diagnosis of IFDs, and the specificity and sensitivity have increased with the development of new radiological techniques [309]. Sassi et al. recently found "the hypodense sign" (corresponding to necrosis caused by angio-invasive aspergillosis) to be 100% specific for invasive aspergillosis in contrast-enhanced CT [310]. In the present work (Paper II) almost a quarter of the patients on whom CT scans were performed due to clinical suspicion of IFD would have received a different diagnosis using the EORTC classification if radiological assessment had been performed by a less specialised radiologist. These findings underline the importance of radiology on the classification of IFDs and, consequently, on the evaluation of the performance of diagnostic fungal markers.

Diagnostics

Paper I

As discussed above, the evaluation of positive microbiological cultures in lung-transplant patients poses a clinical problem. In addition, signs of rejection may be present with symptoms indistinguishable from infection, and treatment for both infection and rejection may have to be started empirically in parallel. HBP, IL-1β and IL-8 seemed to discriminate between infection and rejection which should be further investigated. Several attempts have been made to find a biomarker that can be used to diagnose acute rejection and replace transbronchial biopsies, but none has been found to date with sufficient accuracy [101].

Hellyer et al. evaluated IL-1-β and IL-8 as biomarkers for the exclusion of ventilator-associated pneumonia in 150 medical, surgical and trauma patients. They used similar cut-off levels to those in the present work (Paper I**)** (17 pg/ml compared to 10 pg/ml for Il-1-β and 382 pg/ml compared to 1000 pg/ml for Il-8). However, they attempted to maximise high negative predicted values, leading to a negative predictive value of 96% for both Il-1-β and IL-8 [311]. Both cytokines are excreted by the epithelial lining as well as immune cells, and even recently transplanted lungs seem to have the capacity for cytokine response on the same level as non-transplanted lungs. Moreover, no significant difference was found in the levels of analysed biomarkers in BALF from surveillance bronchoscopies in patients without infection at 3 vs. 6 months after LTx (Paper I), indicating that transplanted lungs are not more or less "immuno-active" 3 months post-LTx than 6 months post-LTx.

Neutrophil counts in BALF were not analysed in this study, which is a short-coming as both HBP and lysozyme are released from activated neutrophils, and IL-8 is an effective neutrophil chemoattractant. However, studies of HBP in cerebrospinal fluid during meningitis, in urine during urinary tract infection, and in plasma during sepsis, have revealed that HBP is a better diagnostic marker of infection than neutrophil count [20, 22, 23]. However, as no neutrophil counts were available, the samples were graded according to the presence of inflammatory cells in transbronchial biopsies and cytology of BALF. All three biomarkers, HBP, IL-8 and IL-1-β, were found to be more sensitive than semi-quantitative grading of inflammation in TBB/cytology for the detection of infection.

Repeated findings of the same microbe were recorded in 30 BALF samples from 8 patients in our first study (Paper I). BALF samples from the same individual with the same microbe were classified as possible, probable and definite infection. However, there was no difference in the distribution of biomarkers compared to patients with pathogens found just once. This might be correlated to the findings presented in Paper III**,** where similar microbes were found in samples with and without signs of infection. Although neither of the study designs allowed complete

analysis of the response to single microbe in individuals, the findings suggest that host factors and microbial interactions could be responsible for the development of lung infection, rather than a specific pathogen *per se*.

Paper II

Prospective studies on biomarker performance in diseases with low prevalence require very high numbers of patients. Given the seriousness of IFD, antifungal treatment must be started without delay, before planned biomarker sampling. Furthermore, multiple parallel treatments, and the often complicated clinical picture, make grading and retrospective evaluation of possible fungal infections very complex. Antifungal treatment was started in 35% of the patients in this study based on clinically suspected IFD, but only half of them were classified as proven, probable or possible cases of IFD according to the EORTC criteria. In spite of these difficulties, some very interesting findings were made regarding biomarkers, in particular BG.

Quantification of BG above the maximum detection limit of the assay and a graphical evaluation of the pattern improved the diagnostic capacity. Steadily increasing values and maximum levels >800 pg/ml were highly indicative of IFD. Other research groups have studied BG dynamics in relation to treatment response to IFD [312-315]; however, to the best of our knowledge, no other studies have investigated the role of BG dynamics in the diagnostic performance of the BG test. Furthermore, high levels of triglycerides in serum gave false-positive BG results, which confirms our previous suspicions [316].

In contrast to our results, older studies report that positive galactomannan precedes other clinical signs of aspergillosis suggesting a capacity for early diagnosis of invasive aspergillosis[317-319]. In our study, the sensitivity of galactomannan was very low at time of diagnosis and did not exceed 57% at any time point in the course of infection. However, the fact that two of these patients were on mold-active drugs at time of diagnosis may have affected the sensitivity negatively[320].

It has been proposed that bm-gliotoxin could be used as a diagnostic marker for invasive aspergillosis [222], and a sensitivity and specificity of 62% and 93% were reported in a recent clinical study [321]. Despite the fact that a similar methodology [222] was used to that in other studies, none of the serum samples from the 15 patients in the present study with proven, probable or possible invasive aspergillosis were bm-gliotoxin-positive. Our inability to reproduce previously published results regarding the detection of bm-gliotoxin in serum from patients with invasive aspergillosis casts doubts on its suitability as a diagnostic marker of invasive aspergillosis.

Previous reports have shown that the DA/LA ratio in urine may be useful in the diagnosis of invasive candidiasis from blood cultures, in particular infections caused by *C. albicans* [226, 227, 322]. In this study, there was only one case of blood-

culture-confirmed invasive candidiasis, caused by a *Candida* non-*albicans* species, and only 3% of over 1800 urinary tests from 130 high-risk patients were positive. This reflects the current epidemiology of IFD in haematological patients, i.e., a decreasing incidence of *C. albicans* infections due to the extensive use of fluconazole prophylaxis [323, 324], and also implies that the occurrence of blood-culture-negative invasive candidiasis is probably very low in this cohort of patients. Thus, the DA/LA ratio is not an important general tool for the early diagnosis of IFD in haematological patients at present.

Nosocomial transmission

Tracing and managing MDR bacteria

The outbreak of Pae-MBL described in Paper IV was first identified when two neutropenic haematology patients died as a result of fulminant sepsis caused by a strain of Pae-MBL. The two patients had occupied the same room on the same ward, one after the other, but had never met, and had not been admitted to the ward simultaneously. Due to the uniqueness of the strain, a relationship was suspected, although admitted to two different hospitals at the time, and environmental screening showed that the bacterium originated in the sink drain of the patient's bathroom. *Pseudomonas* sepsis with a susceptible strain of *Pseudomonas* in two patients, would probably have not triggered the same response. However, the risk of transmission would probably be the same for a susceptible strain, but less noticeable. Several nosocomial transmission paths of MDR bacteria have been revealed in numerous outbreak reports. The relatively low frequency of MDR bacteria in Sweden may have led to lower awareness of nosocomial transmission. However, the low frequency makes tracing easier once an outbreak is suspected.

New-generation sequencing techniques offer completely new possibilities for tracing bacterial strains, and mapping routes of nosocomial transmission through a hospital [325]. However, environmental cultures are not always easy to evaluate, especially when tracing a susceptible strain. Sink drains harbour a massive amount of bacteria, and selective cultures were therefore performed in this work to find the Pae-MBL. Biofilm properties also influence the sensitivity of environmental cultures as bacteria in biofilms may be in a viable but non-culturable state. Bravo et al. recently reported reduced culturability of *Acinetobacter pittii* from various surfaces after 43 days at room temperature (for example, reductions of 81% on plastic and 78% on white coats). However, 86% of the populations regained their culturability, and their biofilm-forming ability was increased, after rehydration in a nutrient medium at 37 °C. [326].

Contamination of sinks

Biofilm-forming capacity is a major virulence factor of *P. aeruginosa*. There appears to be interplay between resistance, virulence and biofilm formation [275], and an association has been found between the ability to form biofilms and higher mortality due to bacteraemia caused by carbapenem-resistant *Pseudomonas* [276]. The strain identified in the current study (Paper IV) had a very pronounced biofilm-forming capacity; indeed the microbiologist said that he could almost spot the strain on the disk without resistance pattern. This probably contributed to the long-term survival of the Pae-MBL strain in the hospital plumbing system.

Only sinks in the patient bathrooms were found to be contaminated with Pae-MBL. This is in contrast to most previous outbreak reports, where hand hygiene sinks used by health care workers have been found to be contaminated [265, 267, 327]. Balm et al. found that hand hygiene sinks used inappropriately (i.e. for the disposal of patient secretions and rinsing of re-usable patient care items) were contaminated more often than sinks that were not misused [265]. Patient bathroom sinks are exposed to patient secretions, and this is the probable path of contamination. Interestingly, 5 out of 12 patients with Pae-MBL identified in the present study had pulmonary disease and Pae-MBL in sputum. Pulmonary disease seems be a risk factor for both transmission to the sink and colonisation/infection from the sink. This should be taken into consideration on wards alternating patients with pulmonary diseases such as cystic fibrosis with susceptible lung-transplant patients. The care of patients harbouring MDR bacteria and patients with neutropenic fever in the same ward may also lead to a higher risk of nosocomial transmission.

Bacterial biofilms are often difficult to eradicate. In this work, acetic acid was found to have an exceptionally good effect on *Pseudomonas* biofilms, which is also supported by a recent publication by Bjarnsholt et al. [292]. They showed that a mature *Pseudomonas* biofilm grown for 3 days in a continuous-flow system could be completely eradicated using acetic acid at a concentration as low as 0.5%. The direct antibacterial effect could be the result of the low pH created by the acid. However, interestingly, they found that other organic acids, creating the same pH, did not exert the same antibacterial effect, indicating that it is not only the low pH that causes bacterial death, but that the acetic acid molecule itself also plays a role [292].

Although an *in vitro* biofilm may be different from long-standing biofilms in water systems, acetic acid seemed to effectively eradicate Pae-MBL from sink drains in the present study. However, Pae-MBL seemed to survive further down the drainage system, and re-emerged after the sinks had been changed and the acetic acid routine discontinued (Paper IV).

A recent publication by Kotay et al. presents a detailed model of bacterial dispersion from sink drain reservoirs. Briefly, bacteria enter the sink drain with

body secretions, e.g. in sputum during teeth brushing. Growth in the sink drain is promoted by nutrients (any organic material other than water), and the bacterial biofilm grows upwards and reaches the strainer. Water from the tap then splashes on the strainer causing dispersal of the bacteria. They also demonstrated the retrograde spread of bacteria from sink to sink through bacterial growth in the drainage pipes [328]. This model could also explain the effect of acetic acid in our intervention, as 250 ml of 24% acetic acted on the biofilm on the strainer and the drainage pipe from the sink, rather than totally eradicating *Pseudomonas* from the U-bend. In fact, cultures 5-7 cm down the sink drainage pipe would be more representative of the strainer than the U-bend.

The findings presented in Paper IV emphasize the importance of appropriate design and cleaning of sinks. Increasing awareness of risks has led to the emergence of sinks designed to minimise the risk of bacterial transmission on the market. The installation of new sinks designed to prevent bacterial transmission is the preferred response, but treatment with acetic acid is a simple and inexpensive method of preventing the transmission of *Pseudomonas* in the absence of better long-term solutions.

Conclusions

The most important conclusions drawn from the studies presented in this thesis are given below.

- HBP, IL-1β and IL-8 could be useful biomarkers for the detection of pulmonary infection in lung-transplant patients. These biomarkers also seemed to discriminate between infection and rejection.
- BG is valuable in the diagnosis of IFD in high-risk haematology patients when sampling is performed later in the course of infection, especially when levels above 400 pg/ml are quantified and the dynamic pattern is evaluated.
- Both BG and galactomannan may serve as tools for exclusion of IFD and invasive aspergilloisis when used as screening markers among high risk hematology patients. However, the results of this work do not support the use of BG nor galactomannan for early diagnosis of IFD, and the diagnostic role of bm-gliotoxin or DA/LA ratio in urine in this cohort of patients seems questionable.
- Elevated triglycerides could be a source of false BG results.
- Qualified radiology assessment is crucial in the diagnosis of IFD.
- Microbial findings in BALF from lung-transplant patients in Sweden are frequent and often poly-microbial.
- Microbiological findings are similar in BALF from patients with and without signs of lung infection underlining the importance of evaluating microbiological findings in the light of clinical symptoms and endobronchial appearance in the assessment of lung infections in lung-transplant patients.
- MDR bacteria in BALF are rare in lung-transplant patients in Sweden.
- Nosocomial transmission of a MBL producing strain of *Pseudomonas aeruginosa* was found to be associated with sink drains, and weekly treatment with acetic acid is proposed as a simple and inexpensive method of preventing transmission.

Future Perspectives

Much remains to be discovered about the complex transmission of bacteria from the environment to patients, and from patients to the environment. For example, it would be of interest to further examine to what extent the Gram-negative bacterial infections seen in ICUs are derived from the environment. Studies including regular microbial surveillance of patients and their environment, including full-genome sequencing, should be carried out. Patient risk factors should be correlated to colonisation and infection, and the routines of health care staff included in the monitoring.

Various questions remain to be answered about the intricate interplay between microorganisms, and between microorganisms and the host. It would be of interest to further examine which factors in the host lung that predispose for lung infection. Could an antimicrobial response in the lung, a cytokine profile or an antimicrobial peptide, be a marker for colonisation rather than infection? Futher studies focusing on the interplay between bacterial communities in the lung and how they interact in the development of lung infection should be carried out. In particular the possibe relation between *Candida* and *Pseudomonas* is intriguing. Studies with repeated sampling and analysis of BALF from long term colonised patients compared to samples from patients with negative microbiology or single findings should be carried out in parallel with *in vitro* examination of bacterial and fungal expression and lung epithelial response.

It would be of great value if HBP or a cytokine profile could determine the appropriate duration of treatment of lung infections. In the present work, a large proportion of the patients with clinically suspected infection had received prior antibiotic or antifungal treatment at the time of bronchoscopy. To be able to determine the effect and duration of treatment, studies should be carried out in which samples are collected before treatment, at regular intervals during treatment, and during a non-infectious period for comparison.

Further studies of the specific antimicrobial and anti-biofilm-forming properties of acetic acid should be carried out. If acetic acid is tolerated by lung epithelial cells, maybe a weak solution, or a modified molecule, could be used for BAL irrigation in complicated MDR *Pseudomonas* lung infections. *In vitro* studies of acetic acid effect on lung epithelium and association of anti-bacterial effect to pH would primarily be needed.

Populärvetenskaplig sammanfattning

Avhandlingens fyra arbeten handlar om infektioner, diagnostik och risk för smitta hos patienter med nedsatt immunförsvar. Antalet patienter med nedsatt immunförsvar ökar då modern behandling av cancersjukdomar, autoimmuna sjukdomar och organtransplantationer som biverkan ger ett nedsatt immunförsvar. Patienter med ett nedsatt immunförsvar löper hög risk att drabbas av infektioner orsakade av både vanliga virusinfektioner som cirkulerar i samhället och ovanliga bakterier och svampar som bara drabbar patienter med nedsatt immunförsvar. Patienter som behandlas för blodcancer löper extra hög risk att drabbas av invasiva svampinfektioner, det vill säga infektioner där mögel och jästsvamp når blodbanan och inre organ. Lungtransplanterade patienter löper stor risk för lunginfektioner genom att den transplanterade lungan ständigt utsätts för mikroorganismer via inandningsluften. För patienter med nedsatt immunförsvar utgör även sjukhusmiljön en risk för infektioner. *Pseudomonas aeruginosa* är en bakterie som associerats till smittspridning på sjukhus och som trivs i fuktiga miljöer till exempel badrum och handfat.

För att ha en chans att behandla infektioner hos dessa utsatta patienter krävs tidig diagnostik och snabbt insättande av korrekt behandling. Att diagnosticera en infektion hos patienter med ett nedsatt immunförsvar är dock ofta svårt efterom patienterna inte uppvisar normala inflammationssymptom. En patient med defekt immunförsvar får till exempel inte feber, påverkan på vita blodkroppar, eller röntgenförändringar på samma sätt som patienter med ett normalt immunförsvar. Hos organtransplanterade kan dessutom symptom vid infektion förväxlas med symptom på att det egna immunförsvaret reagerar mot det transplanterade organet, så kallad rejektion. Säkra markörer för infektion saknas både för bakteriella infektioner och svampinfektioner. Behandlingen får ofta startas vid misstanke om infektion snarare än säkerställd infektion. Många antibiotika och svampmedel är dessutom förknippade med biverkningar och interaktioner med immunsupprimerande läkemedel. För att kunna välja rätt behandling vid misstanke om infektion krävs epidemiologisk kunskap om vilka bakterier, svampar och virus som brukar drabba dessa patientgrupper.

Lungtransplanterade patienter genomgår bronkopskopi flera gånger under det första året efter lungtransplantation, både som rutin och vid symptom på infektion eller rejektion. Vid bronkopskopi genomförs så kallat broncheoalveolärt lavage

vilket innebär att lungan sköljs med vätska som sugs upp och kan analyseras för olika ämnen.

I det första arbetet undersöktes om heparin bindande protein (HBP), cytokinerna IL-1-beta, IL-8, IL-10, TNF, IL-6 och lysozym i så kallat broncheoalveolärt lavage kunde påvisa lunginfektion hos lungtransplanterade patienter. I studien analyserades broncheoalveolärt lavage från lungtransplanterade patienter för Heparin-bindande protein (HBP) som utsöndras av vita blodkroppar, en panel av kroppsegna försvarsmolekyler, cytokiner (IL-1-beta, IL-8, IL-10, TNF, IL-6) och lysozym. HBP har tidigare visat sig vara en bra markör för allvarlig infektion i blod. Undersökningen visade att nivåer av HBP Il-1-beta och IL-8 steg signifikant i broncheoalveolärt lavage vid lunginfektioner men var fortsatt låga vid rejektion. HBP, Il-1-beta och IL-8 skulle därmed kunna användas vid diagnostik av lunginfektioner.

Det andra arbetet syftar till förbättrad diagnostik av invasiva svampinfektioner hos patienter med blodcancer och stamcellstransplanterade patienter. En kombination av nya och tidigare beprövade biomarkörer för invasiv svampinfektion undersöktes. Betaglukan är en komponent i de flesta svampars cellvägg och galaktomannan är en specifik markör för invasiv mögelinfektion i blod. Båda har använts för diagnostik av invasiva svampinfektioner men det saknas konsensus kring deras värde och hur de skall användas. Genom att späda blodproverna kunde vi se att den egentliga maxnivån för betaglukan var betydligt högre hos patienter med invasiv svampinfektion än det tidigare maxvärdet för analysen på 400 ng/ml. Vi kunde också identifiera mönster av betaglukan över tid där de med invasiv svampinfektion hade tydligt stigande värden >800 ng/ml. Underökningen visade att både betaglukan och galactomannan kunde användas för att utesluta invasiv svampinfektion men däremot var ingen av markörerna tillräckligt bra för att användas som screeningprov för att hitta invasiv svampinfektion. Förhöjda triglycerider identifierades som en källa till falskt positivt betaglukan. Diagnostiken av invasiva svampinfektioner bygger till stor del på röntgenfynd. En extern röntgenspecialist i svampdiagnostik eftergranskade alla datortomografiundersökningar med misstänkt svampinfektion enligt den lokala radiologen. Det visade sig att svaren från den lokala radiologen skilde sig från specialistutlåtandet i nästan en fjärdedel av fallen, vilket belyser både svårigheten med diagnostiken och vikten av en kvalificerad röntgenbedömning.

I det tredje arbetet kartlägger vi mikrobiella fynd i bronkeoalveolärt lavage hos lungtransplanterade patienter i Sverige. Lungtransplantationer görs vid två universitetssjukhus i Sverige; Göteborg och Lund. I studien inkluderades samtliga patienter från båda orterna under två års tid. De allra flesta patienterna (85%) hade mikrobiella fynd i broncheoalveolärt lavage, ofta med flera olika mikrober samtidigt. Antalet multiresistenta bakterier var dock lågt. Jästsvamp var vanligt den första månaden medan bakteriefynd ökade under de första tre månaderna och mögelsvamp kom först vid sex månader. *Pseudomonas aeruginosa* var den

bakterie som dominerade både i broncheoalveolärt lavage från patienter med och utan tecken på infektion. De mikrober som fanns i broncheoalveolärt lavage hos patienter med symptom på lunginfektion skilde sig inte från fynd vid bärarskap utan infektion. Resultaten visar på vikten av en sammanvägd bedömning av; symptom hos patienten, hur det ser ut i lungan vid bronkoskopin och mikrobiologiskt fynd, för att ställa diagnosen lunginfektion. Studien ger även viktig epidemiologisk kunskap om vilka mikrober som förekommer hos lungtransplanterade patienter i Sverige och kan användas till att guida profylax och behandling.

I det fjärde arbetet kartlägger vi smittspridning på sjukhuset av en multiresistent stam av *Pseudomonas areuginosa.* Sammanlagt kunde 13 patienter med bakterien identifieras från 2009–2014. Samma bakterie hittades även i 12 handfat på patienttoaletten i de rum där de smittade patienterna vårdats. Jämförelse av bakterier från patienter och handfat visade samma bakteriestam och smitta bedöms ha skett från handfat till patienter. *P. aeruginosa* bildar en skyddande biofilm i den fuktiga miljön i avloppsledningar som är svår att få bort. Vi provade att byta handfaten men bakterien kom tillbaka och kunde även hittas längre ned i avlopp-systemet. Ättika är en gammal hushållsmetod för rengöring och har framgångsrikt används mot *P. aeruginosa* i kroniska sår. Vi undersökte ättikas bioegenskaper och kunde notera att det var exceptionellt effektivt mot den aktuella stammen av *Pseudomonas areuginosa*. Vi hällde då 2,5 dl 24% ättika i handfaten en gång i veckan, odlingarna blev därefter negativa och inga nya patientfall kunde identifieras.

Acknowledgements

Many people have contributed to this work and helped me in completing this thesis. I wish to express my thanks to all of them, and those below in particular.

Bertil Christensson, my first supervisor, who started it all, and who was always there to support me when needed. Thank you for your constructive criticism and for all the good laughs.

Lisa Påhlman, my second supervisor, without whom I wouldn´t have succeeded. Thank you for your constant and unobtrusive support and excellent research abilities. From you, I learnt that less is more, even in research.

My co-supervisor Gisela Otto for her valuable input and comments.

Helena Hammarström my companion and co-worker, with the mind of a true researcher. We shared a lot of late-night emails, telephone calls, and massive Excel files, but most importantly, I want to thank you for your friendship.

The Gothenburg members of the research group: Vanda Friman, Christine Wennerås, Nahid Kondori and Helena: thank you for your good company at conferences your valuable input at meetings, and for the endless telephone discussions. Special thanks to Vanda for her generosity and that productive weekend on Orust.

Johan Richter and colleagues at the Department of Haematology in Lund, all the patients and nurses for their various contributions to this work.

The Lung Transplant and Bronchoscopy Unit in Lund: Lennart Hansson, Ingrid Skoog, Hillevi Larsson, the patients, the lung-transplant team, bronchoscopy staff and transplant co-ordinators; thank you for your invaluable contributions and participation. Special thanks to Hillevi for input and help with the bronchoscopy photographs.

Thanks to Christina Pehrson, for her massive efforts in sample logistics, DA/LA analysis and CRF administration, for always being ready to help. Lennart Larsson and Pavel Markowicz, for their introductions to mass spectrometry and gliotoxin.

Malin Inghammar, for her encouragement, and for making statistics almost understandable.

My co-authors: Eva Melander, Mattias Mörgelin, Karin Sjöstrom, Barbro Olsson Liljequist, Claus Peter Heußel, Jenny Isaksson and Gerdt Riise, for their valuable knowledge and input.

The MOL group at BMC B14, especially Gisela Hovold, for compensating for my lack of laboratory skills. Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, for BG analysis, especially Jenny Isaksson.

Carina Boström and Carina Andersson for their enduring sampling of sink drains and Anita Eriksson for Pae-MBL cultures. Jill Ryd for tirelessly filling in the CRFs.

Göran Jönsson and Fredrik Kahn, for excellent input at my half-way assessment.

All the Heads of Department at my various places of work: Gisela Otto, Peter Lanbeck, Eva Melander, Erik Sturegård and Cecilia Löfgren, for their encouragement and for allowing me time off for research.

Colleagues at the Department of Infectious Diseases in Lund, for many years of training, collaboration and an inspiring atmosphere. Bengt Ljungberg, for his support and years of juggling the duty rosters to fit my complicated logistics. The Department of Infectious Diseases in Lund will always feel like home to me.

Colleagues and nurses at the Department of Hospital Infection Prevention and Control: Anna H, Kristina T, Ulf K, Kalle F and Erik S, for the creative and stimulating working atmosphere.

Colleagues at the Department of Infectious Diseases in Helsingborg; I look forward to being part of your team!

Karin Norlin, my first mentor at the Department of Infectious Diseases in Lund; for being an important role model and inspiration. Thank you for introducing me to our, now joint, project “Vattentankar i Kenya”.

Friends of all kinds, for their faithful friendship and encouragement. For being there to the bitter end! Special thanks to Johanna S, for our invaluable walks, Elisabeth C, for her honest, lasting friendship, and Anna H, for her constant support and close friendship, no matter what!

My extended family, cousins with families, aunts and the big Aspelund clan. I am grateful to be a part of this family!

My brother Johan, for his unconditional loyalty, Marie and my lovely nieces.

Mamma Elisabeth, for her unconditional love, support and flexibility in every possible way. I wish Pappa were here.

Johan, Folke, Ivar and Jarl, my greatest treasures, for being just the way you are. Johan, for your constant support, for heating milk to my coffee and for your love.

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Printed by Media-Tryck, Lund 2018 NORDIC SWAN ECOLABEL 3041 0903

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FACULTY OF MEDICINE

Division of Infection Medicine
Department of Clinical Sciences Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2018:27
ISBN 978-91-7619-594-9
ISSN 1652-8220