PULMONARY COMPLICATIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Ulrica Forslöw

Stockholm 2011
Learning without thought is labor lost; thought without learning is perilous.

Confucius
ABSTRACT

The respiratory tract is one of the most common and serious sites for complications in HSCT patients. In this project the incidence, outcome, and risk factors for patients with infectious or non-infectious pulmonary complications were studied. Bronchoscopy and pulmonary function tests (PFTs) were evaluated as diagnostic tools.

Between 1975 and 2003, pneumonia was found to be the most common cause of early death (within 100 days) after HSCT. The cumulative incidence was 5.6% compared to 10% for all other causes. However, this three-decade study exhibited a striking change over time: the cumulative incidence of early death due to pneumonia decreased from 8.9% in the first decade studied to 2.8% in the third decade. In the fourth study (2000-2009) this incidence was 3.2%. However, death from overall pneumonia (early and late pneumonia) was 10.5%, indicating that pneumonia is still a common cause of death. Bronchoalveolar lavage (BAL) was shown to be a safe and useful diagnostic tool to establish the causative pathogens of pneumonia: this procedure contributed to the diagnosis in 43 of 68 (63%) episodes of pneumonia in the second study (1998-2004). BAL was especially important for establishing pneumonia due to Aspergillus and Cytomegalovirus. Comparing the results of other culture specimens, these pathogens would not have been found pre-mortem without this procedure. In 42 (62%) cases of pneumonia, the treatment was either changed or continued according to the BAL results. PFTs are also important diagnostic tools. We considered FEV₁ as the most important parameter for detection and monitoring the development of BO, a progressive and persistent non-infectious complication characterized by airflow obstruction. Furthermore FEF₇₅ was reduced in 28% patients with BO and thus may serve as an early warning. Patients who developed BO late (> 1 year after HSCT) had a better five-year survival than those with an early onset BO.

Statistical analyses revealed that risk factors for early pneumonia death also changed over time. Receiving a T-cell depleted (TcD) graft was identified as a risk factor in the first study (p<0.001). However, this immunosuppressive strategy was abandoned in the early 1990s, due to reports of increased risk of relapse, graft rejection, and infections. During the last decade, other strategies have been increasingly used, to either facilitate engraftment, suppress the recipient’s own immune system, prevent relapse, graft failure or Graft-versus-host disease (GVHD) while maintaining, if possible, the graft-versus-leukemia (GVL) effect. Such strategies include reduced intensity conditioning (RIC), treatment with donor lymphocyte infusion (DLI), and treatment with mesenchymal stem cells (MSCs). RIC was shown to reduce significantly the cumulative incidence of early death from pneumonia compared to myeloablative conditioning (MAC) (2.1% and 4.2%, respectively) and DLI treatment to have a potentially protective role for BO. However, MSC treatment was associated with overall pneumonia death.

In conclusion, early death due to pneumonia has decreased in the past decades. We believe that new diagnostic and prophylactic strategies and treatments as well as supportive care have been of utmost importance for this improved outcome. For BO patients, DLI seemed to have a protective role. However, because some of the new strategies, such as MSCs may also increase the risk of pneumonia, they should be used with caution. Diagnostic tools such as bronchoscopy and PFTs help determine the etiology of pneumonia (BAL) and detect and monitor BO (PFT) at an early stage. Therefore, these tools should be used as early and correctly as possible.
LIST OF PUBLICATIONS


II. The clinical importance of bronchoalveolar lavage in allogeneic SCT patients with pneumonia. Forslöw U, Remberger M, Nordlander A, Mattsson J. Bone Marrow Transplantation 2010; 45 (5): 945-950.

III. Donor lymphocyte infusion may reduce the incidence of bronchiolitis obliterans after allogeneic stem cell transplantation. Forslöw U, Mattsson J, Gustafsson T, Remberger M. Biology of blood and marrow transplantation 2011; 17 (8): 1214-1221.

IV. Reduced intensity conditioning reduces the risk of early death from pneumonia following hematopoietic stem cell transplantation. Forslöw U, Mattsson J, Remberger M. Submitted.
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<td>AFO</td>
<td>Airflow obstruction</td>
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<tr>
<td>aGVHD</td>
<td>Acute GVHD</td>
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<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
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<td>ALL</td>
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<td>AM</td>
<td>Alveolar macrophages</td>
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<td>ANC</td>
<td>Absolute neutrophil count</td>
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<td>Antigen presenting cells</td>
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<td>Bronchoalveolar lavage</td>
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<td>BcR</td>
<td>B-cell receptor</td>
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<tr>
<td>BM</td>
<td>Bone marrow</td>
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<td>Bone marrow donor worldwide</td>
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<td>Bone marrow transplantation</td>
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<td>BO</td>
<td>Bronchiolitis obliterans</td>
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<td>Central nervous system</td>
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<td>CP</td>
<td>Chronic phase</td>
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<td>Complete remission</td>
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<td>Cyclosporine A</td>
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<td>Diffusing capacity for carbon monoxide</td>
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<td>Donor lymphocyte infusion</td>
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<td>Expiratory residual volume</td>
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<td>Forced expiratory flow</td>
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<tr>
<td>FEV</td>
<td>Forced expiratory volume</td>
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<td>FFB</td>
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<td>Fludarabine</td>
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<td>FRC</td>
<td>Functional residual capacity</td>
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<td>Forced vital capacity</td>
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<td>Description</td>
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<tr>
<td>rTBI</td>
<td>fractionated TBI</td>
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<td>GF</td>
<td>Graft failure</td>
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<td>Gy</td>
<td>Gray</td>
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<td>G-CSF</td>
<td>Granulocyte colony-stimulating factor</td>
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<td>GVHD</td>
<td>Graft-versus-host disease</td>
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<tr>
<td>GVL</td>
<td>Graft-versus-leukemia</td>
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<td>HC</td>
<td>Hemorrhagic cystitis</td>
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<td>Human herpes virus 6</td>
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<td>HI</td>
<td>Haemophilus influenzae</td>
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<td>Human leukocyte antigen</td>
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<td>HRCT</td>
<td>High resolution tomography</td>
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<td>HSC</td>
<td>Hematopoietic stem cell</td>
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<td>Hematopoietic stem cell transplantation</td>
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<td>Herpes simplex virus</td>
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<td>Herpes zoster virus</td>
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<td>IFD</td>
<td>Invasive fungal disease</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<td>Interleukin</td>
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<td>IFNγ</td>
<td>Interferon gamma</td>
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<td>IPS</td>
<td>Idiopathic pneumonia syndrome</td>
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<tr>
<td>i.v</td>
<td>Intravenous</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>MAC</td>
<td>Myeloablative conditioning</td>
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<td>MCP-1</td>
<td>Monocyte chemoattractant protein-1</td>
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<td>MEF</td>
<td>Maximal expiratory flow</td>
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<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>MMF</td>
<td>Mycophenolate mofetil</td>
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<td>MRD</td>
<td>Minimal residual disease</td>
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<tr>
<td>MSC</td>
<td>Mesenchymal stem cell</td>
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<tr>
<td>MTX</td>
<td>Methotrexate</td>
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<tr>
<td>MUD</td>
<td>Matched unrelated donor</td>
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<td>MVV</td>
<td>Maximal volume ventilation</td>
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<tr>
<td>NC</td>
<td>Nucleated cell</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NK cell</td>
<td>Natural killer cell</td>
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<tr>
<td>NTM</td>
<td>Non-tuberculous mycobacterium</td>
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<tr>
<td>OKT-3</td>
<td>Orthoclone, monoclonal antibody against CD3</td>
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<tr>
<td>PB</td>
<td>Peripheral blood</td>
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<tr>
<td>PBL</td>
<td>Peripheral blood leukocytes</td>
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<td>PBSC</td>
<td>Peripheral blood stem cell</td>
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<td>PCP</td>
<td>Pneumocystis pneumonia</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PEF</td>
<td>Peak expiratory flow</td>
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<tr>
<td>PFT</td>
<td>Pulmonary function test</td>
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<td>PTLD</td>
<td>Post-transplant lymphoproliferative disease</td>
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<tr>
<td>PUVA</td>
<td>Psoralen and ultraviolet A light</td>
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<tr>
<td>RIC</td>
<td>Reduced intensity conditioning</td>
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<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
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RV  Residual volume
SAA  Severe aplastic anemia
SCID Severe immunodeficiency disease
Sec  Second
Spp  Species
TBB  Transbronchial biopsy
TBI  Total body irradiation
TcD  T-cell depletion
TcR  T-cell receptor
Th   T-helper
TNF-α Tumor necrosis factor alpha
TLC  Total lung capacity
TLI  Total lymph node irradiation
TRM  Transplant related mortality
T-reg cells  T-regulatory cells
TV   Tidal volume
VC   Vital capacity
VP   Vepecide
VOD  Veno-occlusive disease
1 THESIS SUMMARY

Over the last fifty years, hematopoietic stem cell transplantation (HSCT) has become the preferred therapy for patients with life-threatening diseases in the lymphohematopoietic system. However, HSCT is associated with a variety of complications that threaten the recovery of the transplant recipients. Because the respiratory tract is one of the most common and serious sites for complications in HSCT patients, this project was performed to evaluate the incidence, outcome, and risk factors for patients with infectious or non-infectious pulmonary complications. Furthermore, bronchoscopy and pulmonary function tests (PFTs) were evaluated as diagnostic tools for pneumonia and bronchiolitis obliterans (BO), two diagnoses that may cause considerable morbidity and mortality after HSCT.

Between 1975 and 2003, pneumonia was found to be the most common cause of early death (within 100 days) after HSCT. The cumulative incidence was 5.7% compared to 10% for all other causes. However, this three-decade study exhibited a striking change over time: the cumulative incidence of early death due to pneumonia decreased from 8.9% in the first decade studied to 2.8% in the third decade. This result agreed with the fourth study, covering the years between 2000 and 2009, in which the cumulative incidence of pneumonia was 3.2%. However, in this study death from overall pneumonia (early and late pneumonia) was 10.5%, indicating that pneumonia is still a common cause of death. Clearly, pneumonia is important to diagnose and to prevent at an early stage. Bronchoscopy with bronchoalveolar lavage (BAL) was shown to be a safe and useful diagnostic tool to establish the causative pathogen or pathogens of pneumonia: this procedure was found to have contributed to the diagnosis in 43 of 68 (63%) episodes of pneumonia in the second study (1998-2004). BAL was especially important for establishing pneumonia due to *Aspergillus* and *Cytomegalovirus* (CMV). Comparing the results of other culture specimens, these pathogens would not have been found pre-mortem without this procedure. In 42 (62%) cases of pneumonia, the treatment was either changed or continued according to the BAL results, and thus the patients could be spared a potentially toxic and unnecessary multidrug treatment.

Another important diagnostic tool is pulmonary function tests. We considered FEV\textsubscript{1} as the most important parameter for detection and monitoring the development of BO, a progressive and persistent non-infectious complication characterized by airflow obstruction (AFO). Dynamic spirometries are rather easy to perform, even in children. FEF\textsubscript{75} is another parameter that was shown to be reduced in seven of 25 (28%) patients with BO and thus may serve as an early warning of BO. Patients who developed BO late (> 1 year after HSCT) had a better five-year survival than those with an early onset BO. This difference is probably due to a more slowly progressing disease and to a better immune competence late compared to early after HSCT.

Thorough statistical analyses were performed to study risk factors for early and overall pneumonia and for BO. Risk factors for death from early pneumonia have also changed over time. Receiving a T-cell depleted (TcD) graft was identified as a risk factor in the first study (p<0.001). However, TcD as an immunosuppressive strategy was abandoned at the Center of Allogeneic Stem Cell Transplantation (CAST) in the early 1990s, due to reports of increased risk of relapse, graft rejection, and infections. During the last decade, other strategies have been either developed or increasingly used, to either facilitate engraftment, suppress the recipient’s
own immune system, prevent relapse of the malignant disease, or prevent graft failure or Graft-versus-host disease (GVHD) while maintaining, if possible, the graft-versus- leukemia (GVL) effect. Such strategies include reduced intensity conditioning (RIC) regimens, treatment with donor lymphocyte infusion (DLI), and treatment with mesenchymal stem cells (MSCs). In these studies, RIC was shown to reduce significantly the cumulative incidence of early death from pneumonia compared to myeloablative conditioning (MAC) (2.1% and 4.2%, respectively). DLI treatment was shown to have a potentially protective role for BO. However, MSC treatment is associated with overall pneumonia death. In Study I (1975-2003) and study IV (2000-2009), bacteremia was identified as another risk factor for both early and overall pneumonia death. In patients who received RIC, bacterial etiology was slightly more common as the cause of early death than in MAC-patients, even if fungal and viral etiologies dominated.

In conclusion, early death due to pneumonia has decreased in the past decades. We believe that new diagnostic and prophylactic strategies and treatments as well as supportive care have been of utmost importance for this improved outcome. For BO patients, DLI seemed to have a protective role. However, because some of the new strategies, such as MSCs may also increase the risk of pneumonia, they should be used with caution. Diagnostic tools such as bronchoscopy and PFTs help determine the etiology of pneumonia (BAL) and detect and monitor BO (PFT) at an early stage. Therefore, these tools should be used as early and correctly as possible.
2 INTRODUCTION

2.1 GENERAL BACKGROUND

2.1.1 Allogeneic hematopoietic stem cell transplantation

Over the past 50 years, hematopoietic stem cell transplantation (HSCT), also known as bone marrow transplantation (BMT), has become a well-established therapy to treat two categories of medical conditions by replacing an abnormal lymphohematoproliferative system with a normal one (1). The first category includes non-malignant diseases that result in bone marrow (BM) dysfunction or dysfunction of BM-derived cells such as aplastic anemia and immunodeficiency syndromes, and genetic diseases such as mucopolysaccharidoses, glycoprotein storage diseases, or the hemoglobinopathies of thalassemia and sickle cell anemia (2). The second category is more common and mainly consists of hematopoietic as well as other malignancies such as solid tumors (3, 4). There are two types of HSCT: autologous and allogeneic. Autologous is when the recipient is also the donor. Allogeneic refers to hematopoietic stem cells collected from another individual and then transplanted into the patient. For allogeneic HSCT, a stringent matching of human leukocyte antigen (HLA) between the donor and recipient is usually required to minimize the risk of graft rejection and to reduce the risk of graft-versus-host disease (GVHD), where immuno-competent cells in the graft cause immune-mediated injury to the patient (5).

2.1.2 Historic landmarks

The first stumbling block, and, of course, unsuccessful steps in trying to transplant bone marrow, were performed in 1891 when Brown-Sequard and d’Arsonaval administered BM orally to a patient with leukemia. However, in 1949 Jacobson and his colleagues managed to show that shielding of a hematopoietic organ could prevent the death of mice that received lethal doses of total body irradiation (TBI) and that injection of spleen cells could further protect from death (6). In the early 1950s, other research groups reported that transfer of BM could be used to protect not only animals, such as mice, but also human cells from lethal irradiation (7-11). In 1957, Donnall Thomas, who in 1990 received the honorable Nobel Prize for his work, performed the first allogeneic BMT in six patients with hematological malignancies, leading to a transitory engraftment in two patients (increase of absolute neutrophil count (ANC) > 0.5 x 10⁹/L after HSCT) (12). These patients were not cured, but this work proved that donor stem cells could develop into hematopoietic cells in a recipient. However, until 1968, several unsuccessful BMT trials were performed. The patients died due to different complications, such as GVHD, rejection, or infections. In the late 1960s and early 1970s, studies of dogs clarified that a successful persistent engraftment could not be performed without a suppression of the recipient’s immune system (13-15). In 1968, Gatti and colleagues and Bach with co-workers performed successful BMTs in two patients with non-malignant diseases. These patients, who both were cured, had HLA-matched sibling donors (16-17). However, the natural lack of matched-related donors raised the question whether unrelated donors could also be used for BMT. Several trials with unrelated donors were performed in the early 1980s, but the severity of GVHD was high in patients with HLA incompatibility (18-19). Gradually, registers were established in many countries and consolidated into a worldwide network. The current number of volunteer donors and cord blood units registered in the Bone Marrow Donor Worldwide (BMDW) database is about 18 million unrelated donors, making it much easier to find an HLA-matched donor. In Sweden, the first BMT was performed at Huddinge Hospital in 1975 (20). As of today (2011-06-10), we have
performed almost 1600 HSCTs at the Center of Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, Huddinge.

2.1.3 The hematopoietic stem cell

The human hematopoietic stem cell (HSC) is a pluripotent cell that has a regenerative capacity and the ability to home to the marrow space after intravenous infusion. This cell, which expresses CD34, is naturally found in 1.8% ± 0.9% of the CD34-expressing BM cells (21, 22). In humans, only a few percent of a donor’s marrow is needed to replace the entire lymphohematopoietic system of a recipient. Following intravenous (i.v) infusion, a high percentage of the HSCs are retained in the marrow due to interactions between adhesion molecules at vascular cells and integrins expressed on the HSCs’ surfaces (23). All cellular elements in the blood derive from the HSC. HSC either develops to lymphatic progenitor cells, from which T- and B-lymphocytes derive, or develops to the myeloid progenitor, from which granulocytes (neutrophils, eosinophils, and basophils), monocytes, erythrocytes, and megakaryocytes may derive.

![Figure 1. The hematopoietic stem cell.](image)

2.1.4 Hematopoietic stem cell sources

In the past, bone marrow was the only source of HSCs for transplants, as there are ten times more of them in BM than in peripheral blood (PB) (22, 24). Recently, other methods to obtain HSCs for transplantation have been developed. However, in patients with non-malignant diseases and in children, BM is still the source of choice due to the lower risk of GVHD and better survival with BM than with other stem cell sources. BM is obtained by aspirations of marrow to a volume of 10-15 ml/kg (500-1500 ml in total) from the donor’s anterior or posterior crista iliaca under general or spinal anesthesia. Next, the BM is filtered to remove bone spiculaes and fat and perhaps other cells such as T-cells, red cells, and tumor cells. The filtered marrow is then infused into the patient. The number of nucleated cells (NCs) should be > 3×10^9/ kg body weight of the recipient. A lower NC dose is associated to an increased transplant related mortality (TRM) (24-25) and risk of infections, hemorrhage, or graft failure within three months after HSCT (26).
HSCs also circulate in the peripheral blood; however, there are only about 0.2 ± 0.1% CD34+ cells (22). If the donors are treated with cytokine granulocyte colony stimulating factors (G-CSF) for four to five days, a sufficient amount of cells may be mobilized from the BM to the PB (27-28) and then separated and gathered by single or multiple leukapheresis. The exact mechanism is still unknown, but it is speculated that G-CSF may activate neutrophil enzymes that proteolytically cleave adhesion molecules, allowing release of HSCs (29). The total blood volume processed is usually 10-15 L, which is 2-3.5 times the donor’s blood volume. After the HSCs are harvested from the donor, they are transplanted into the recipient by an intravenous infusion. However, it should be noted that the HSCs only constitute a small fraction of the cells infused, and that the majority of cells are neutrophils. The first successful HSCT using peripheral blood stem cells (PBSCs) was performed in the early 1980s (30). Since then, an increasing number of HSCTs using PBSC has been seen worldwide. The first studies on how to use PBSC for allogeneic HSCT were published in 1995 (31-32) Today, more than 50% of the HSCTs are performed with PBSC. The advantage with PBSC compared to BMT is that it is less complicated for the donor and that there is no need for general anesthesia. Furthermore, it has been associated with a faster engraftment, since more stem cells can be collected with this method than with BMT (33). However, the disadvantage is that PBSC may be connected with an increased risk of chronic graft-versus-host disease (cGVHD) (34-36).

During the past decade, umbilical cord blood (CB) has also become valuable as a stem cell source. The first transplantation with the use of CB was performed in 1988 (37). The advantages with CB include rapid availability. Today, there are many CB banks worldwide; the first one established was the New York Blood Bank (38). Another advantage of CB is that it has fewer T-cells than PBSC and BM, and the T-cells in CB grafts are mainly naïve, leading to an increased tolerability of HLA mismatching and also a lower risk of GVHD. Cord blood transplantation however, is associated with a slower engraftment, delayed immune reconstitution and an increased risk of graft failure compared to the other stem cell sources (39). The major disadvantage is that CB collections usually do not contain enough HSCs to achieve hematopoietic recovery in adult recipients. Many studies have shown that a NC dose of more than 2 × 10^7/kg is associated with superior engraftment and survival in children (40, 41).

In addition, fetal tissue recently has become an interesting source of HSC due to its immunologic immaturity that allows for a higher degree of mismatching between donor and recipient. However, because of ethical and legal considerations (42), this stem cell source has only a limited place in human HSCT. The first successful transplantation, in which fetal liver cells were given to an infant with severe immunodeficiency disease (SCID), was reported in 1975 (43). Until today, fetal tissues have only been used in a few in utero transplantations at our hospital (44). As of today, BM, PBSC, and CB have been the source of HSCs at Karolinska University Hospital Huddinge in 900, 619, and 58 allogeneic HSCTs, respectively.

### 2.1.5 Conditioning regimens, principles

Before HSCT, the patient receives pre-treatment therapy (conditioning) to eradicate as many of the malignant cells as possible. This treatment aim to provide space for engraftment through a diminished tumor-load and to prevent relapse of the malignant disease (45). In addition, the treatment attempts to suppress the recipient’s immune system to prevent graft rejection, which is the primary aim in all patients with non-malignant diseases. After transplantation, monocytes
are the first cells to engraft, followed by granulocytes, platelets, and natural killer (NK) cells (45).

There are two principles of conditioning regimens, myeloablative conditioning (MAC) and reduced intensity conditioning (RIC). MAC is highly toxic and consists of high doses of chemotherapy with or without TBI as a single dose (8-10 Gy) or as fractionated with doses ranging from 11-15 Gy (46-47). RIC is based on lower doses of chemotherapy in different combinations, sometimes combined with a low dose of TBI. This kind of regimen is less toxic and thus it can also be tolerated by elderly patients or patients with organ dysfunction (48-49). In RIC, the main purpose is to suppress the patient’s immune system by a milder conditioning, facilitating engraftment of the transplant. The anti-leukemia effect is mainly determined by the graft itself, not by the conditioning therapy. The gradual and increasing interest of this less intensive regimen began with reports showing that TRM was reported higher with higher cytoreductive regimens and TBI doses (50-51), and because it was found that patients suffering from GVHD had less relapse of their underlying malignancy (52-53) due to the graft-versus-leukemia (GVL) effect.

2.1.6 Immunosuppressive strategies

Post transplantation, immunosuppressive medication is administered to prevent GVHD and graft rejection, since the effect of the conditioning is not strong or long-lasting enough. If HSCT is performed because of a hematological malignancy, a mild GVHD is needed due to the GVL effect and therefore the immunosuppressive drugs are preferably discontinued within three to six months. A prolonged treatment may lead to an increased risk of relapse (54). Because patients with non-malignant diseases gain no benefit from GVHD, these patients instead receive immunosuppressive drugs for one to two years as a protection against graft rejection (55). Today, the most commonly used treatment is Methotrexate (MTX) together with Cyclosporine A (CsA) (56-58). CsA combined with prednisolone is another alternative when less toxicity is preferred (59). Recently, tacrolimus, mycophenolate mofetil (MMF), and rapamycin (also called sirolimus) are immunosuppressive drugs that have also been used. These drugs, including CsA, suppress the activation and differentiation of naïve T-cells and all immune responses that require activated T-cells by inhibiting interleukin (IL)-2 production. Furthermore, T-cell depletion (TcD), meaning that donor T-cells in the graft are removed before transplantation, has been another approach since T-lymphocytes are necessary for the development of GVHD (60). TcD was previously accomplished by physical techniques (lectin separation and E-rosettes) or the use of antibodies directed against T-cells (61). Another approach more recently used is CD34 separation by using e.g. CliniMacs. However, even if TcD in vivo has shown to decrease the incidence of GVHD, it unfortunately also increases the incidence of relapse, graft rejection, and infections (61-64). Less effective but without some of the disadvantages of TcD is the administration of T-cell antibodies, such as antithymocyte globulin (ATG) or Orthoclone (OKT)-3, given as a part of the conditioning (65-68).

2.2 BASIC IMMUNOLOGY

The task of the human immune system is to protect us from foreign organisms, such as infectious pathogens. This system is divided into two types of host defense: the innate immune system, which is non-specific and responds immediately; and the adaptive immune system, which is specific and responds more slowly. These two systems interact so that signals from the innate system initiate activation of the adaptive effector mechanisms (69).
2.2.1 The innate immune system

The innate system includes barrier protection, such as skin and mucosa, cilia of the respiratory tract, endogenous bacterial peptides, and the normal flora (70-71). The cells involved in this non-specific defense include both phagocytic cells (monocytes/macrophages, neutrophils and dendritic cells (DCs)) and cells that kill pathogens through release of inflammatory mediators (eosinophils, basophils and mast cells). Other important cells involved in the innate system are platelets. Platelets include granulae with cytokines and chemokines and NK cells that can destroy infected or malignant cells by either antibody-dependent cellular cytotoxicity or by cytolytic activity, a process that is controlled by a balance between killing-activating and killing-inhibitory receptors (72).

2.2.2 The adaptive immune system

The cells involved in the adaptive system are antigen-presenting cells (APCs), such as DCs and macrophages, and T-lymphocytes/cells or B-lymphocytes. DCs are the most potent of the APCs. B-cells develop in the BM and migrate to secondary lymphatic tissue. B-cell receptors (BcRs), have a structure similar to the immunoglobulins (Ig) and recognize antigens in their naïve status. Upon activation, B-cells mature into Ig producing plasma cells or memory cells. T-cells, on the other hand, develop in the thymus. The T-cell receptor (TcR) recognizes a complex formed by the self major histocompatibility complex (MHC) molecules and processed antigens (73). MHC and HLA were first described in 1958 by Dausset et al (74). These molecules are encoded by highly polymorphic genes located on chromosome 6 and can be divided into two classes. The three main types of MHC class I molecules include HLA-A, HLA-B and HLA-C and are expressed in all human nucleated cells. The three main types of MHC class II genes include HLA-DR, HLA-DQ and HLA-DP genes, whose expression are restricted to cells of the immune system (75). There are two main subsets of T-lymphocytes; one is defined by the expression of CD4 glycoprotein on its surface and the other by expression of the CD8 glycoprotein. These molecules are co-receptors that give name to two kinds of T-cells. These T-cells can differentiate into effector or memory T-cells, have different functions and can deal with different types of pathogens. This differentiation occurs when the correct antigen is encountered. The antigen presenting DCs migrate to the lymph nodes, where they make contact with naïve CD4 or CD8 T-cells in order to find the right T-cell specific for the antigen presented (76). When the correct T-cell is bound to the antigen-MHC complex, the character of a second signal as well as cytokine production will determine which profile of the T-cell response will be induced. The second signal delivers through a cell-surface protein on the T-cells called CD28, which interact with B7.1 (CD80) or B7.2 (CD86) molecules on the APCs (77-78). Expression of B7 molecules is induced by bacterial breakdown in the macrophages and thereby release of bacterial lipopolysaccharides (LPS). These signals, which are driven by the cytokine IL-2, are essential to induce proliferation and further differentiation of the T-cell, leading to the acquisition of effector function. Another receptor on the T-cell is CTLA4, which binds B7 twenty times more strongly than does CD28. CTLA4 functions as an antagonist; that is, it dampens activation and limits cell activation. In murine models, CTLA4 Ig treatment can ameliorate the lethality of GVHD (79).

The activation of naïve CD8 T-cells, that respond to peptide-MHC class I complexes leads to differentiation into cytotoxic effector T-lymphocytes (CTL), whose main function is to kill cancer cells or cells that have become infected with a virus or some other intracellular pathogen. The activation of naïve CD8 cells to CTLs generally requires a stronger co-stimulatory activity
than needed to activate CD4 T-cells. In circumstances in which the APC offers suboptimal co-stimulation, CD4 cells can help to activate naïve CD8 cells. The general function of CD4 T-cells that respond to peptide-MHC class II complexes is to help other cells of the immune system respond to extra cellular sources of infection. Different aspects of this response are carried out by two subclasses of CD4 T-cells, T-helper cells: (Th)1 and Th2 (80). The cytokines secreted by Th1 cells lead to macrophage activation, inflammation and the production of opzonising antibodies that enhance the phagocytosis of pathogens (cell-mediated immunity). The cytokines secreted by Th2 cells lead mainly to B-cell differentiation and the production of neutralizing antibodies, which bind to extra cellular bacteria and virus particles (humoral immunity) (81, 82). Activated effector T-cells differ from resting naïve T-cells in the types of molecules present at the cell surface and in their abundance. One of the major changes is that they can respond to the specific antigen without the need for co-stimulation via B7-CD28 interaction, which means that they can respond to antigens on cells other than APCs.

2.3 GENERAL COMPLICATIONS AFTER HSCT

2.3.1 Toxic side effects

Treatment with especially myeloablative conditioning may cause damages to several organs. Nearly all patients have mucosal damage in the gastrointestinal tract, paving the way for different infections. The heart is vulnerable to both irradiation and chemotherapy, especially cyclophosphamide (Cy) in doses above 120 mg/kg Cy (83). The liver can be damaged by the conditioning therapy and several drugs used in HSCT such as MTX, CsA, ATG, and some antibiotics (84). A feared toxic side effect of the liver is veno-occlusive disease (VOD) that occurs early after HSCT. VOD has been associated with both irradiation, contraceptives and the use of busulfan (Bu) and is characterized by hepatomegaly, jaundice, ascites, and abdominal pain (85-87). Furthermore, renal insufficiency is common and often caused by nephrotoxic drugs such as CsA, tacrolimus, acyclovir, ganciclovir, amphotericin B and some antibiotics such as aminoglycosides (88). Hemorrhagic cystitis (HC) is also a side effect of the conditioning regimen, often associated with Bu and Cy (89). However, most mild HCs resolve by themselves while severe forms are associated with considerable morbidity and mortality. Neurological toxicity and alopecia may also occur (90-91). The lungs are especially vulnerable and may be damaged despite shielding to decrease the irradiation dose (92). However, studies have shown a significantly lower pulmonary TRM with than without lung shielding (93). Cytotoxic drugs such as Cy, Bu, and MTX may also cause lung injury (94).

2.3.2 Immune-mediated side-effects of HSCT

2.3.2.1 Graft-versus-host disease

GVHD is the main complication after HSCT. There are two distinctive forms of GVHD based on the time of onset and clinical features — the acute (in general within 100 days after HSCT) and the chronic (in general after 100 days after HSCT). However, overlap syndromes exist, since acute GVHD (aGVHD) can occur later than 100 days after HSCT in patients given non-myeloablative conditioning (95-96). The incidence of aGVHD varies between 40-70% and for cGVHD between 28-100% for all allogeneic transplanted patients (97).

Acute GVHD is a rapidly proceeding systemic illness, manifested by damage to the skin, liver, and/or gastrointestinal mucosa. Depending on the severity, aGVHD is graded from 0-IV, where 0 means no GVHD and IV means severe damage to one or more of these organs (98). The un-
derlying pathophysiology of aGVHD is divided into three phases: 1) the tissue damage attributable to conditioning; 2) the donor T-cell activation; and 3) the immune-based host tissue damage (99-100). In the first phase, bacterial products such as LPS and CpG (cytosine guanine) DNA translocates into the circulation due to the barrier damage (101). This promotes an inflammatory response in the recipient, characterized by release of IL-1 and tumor necrosis factor alpha (TNF-α) and IL-6, mainly from cells in the innate immune system. This inflammatory response leads to the second phase by the expression of MHC molecules and other costimulatory molecules on both donor and host APCs, required for activation of donor T-cells. The following proliferation and differentiation leads to Th1 cells and thus production of inflammatory cytokines (mainly TNF-α, interferon-gamma (IFN-γ), and IL 1-2). These cytokines and other activated effector cells, such as CTLs and NK cells, cause the third phase with tissue damage.

Chronic GVHD (cGVHD), on the other hand, resembles autoimmune syndromes in that it damages the tissues and organs by the Th2 pathway and produces symptoms such as keratoconjunctivitis, dermatitis, liver dysfunction, gastrointestinal dysfunction with malabsorption, and pulmonary insufficiency (5, 102). Chronic GVHD usually develops between three and twelve months after HSCT and is usually but not always preceded by aGVHD. Chronic GVHD may be classified as limited or extensive (103). The overall severity is defined as mild, moderate, or severe (104). In limited cGVHD, the skin and/or the liver are involved. Severe cGVHD means that there is an involvement of further tissues or organs with a strong negative effect on clinical performance status. Chronic GVHD is also associated with prolonged immunodeficiency, which may lead to recurrent and sometimes fatal infections (104).

For a long time, IL-2 has been thought to be the primary cytokine involved in the pathogenesis of GVHD. Initial studies showed intensified manifestations of GVHD when exogenous IL-2 was administered (105). However, later studies have demonstrated an inhibition of donor CD4 T cells’ GVHD-producing activity while simultaneously preserving the GVL-effect of the donor CD8 T-cells when IL-2 is administered in vivo (106-107). This protective effect seems to depend on the induction of T regulatory (Treg) (CD4+ CD25+) cells and/or Th17 function (108-110). Th17 is a pro-inflammatory cytokine that is mainly produced by a distinct lineage of CD4 cells and may be associated with the development of autoimmune diseases (111-112) since there is a reciprocal regulation of Th1 and Th17 responses. Th17 differentiation is suppressed by the Th1 cytokine IFN-γ (113) and if there is no IL-17, a potentiated Th1 profile develops, leading to an increase in GVHD mortality (114).

Treatment of GVHD is primarily based on high doses of steroids often in combination with CsA. Other drugs used are azathioprin, thalidomide, and different antibodies such as ATG, OKT-3, and IL-2 antibodies (115-117). Using psoralen and ultraviolet A light (PUVA) as therapy for cutaneous GVHD is sometimes effective (118-119). Recently, IL-2 receptor monoclonal antibodies (basiliximab and daclizumab) have also been used (120-121) as has transplantation of mesenchymal stem cells (MSCs) for patients with severe aGVHD (122). To date, 102 treatments with MSCs have been performed at CAST, and 41 of these have been part of randomized studies. However, despite powerful immunosuppressive medication, the outcome in patients with severe acute GVHD is poor and treatment failures and severe infectious complications are common.
2.3.2.2 Graft-versus-leukemia effect

The discoveries that patients who do not have GVHD have an increased risk of leukemia-relapse and a low grade of GVHD could be an advantage for the recipient led to the discovery of the GVL or graft-versus-tumor (GVT) effect (52). Studies have shown that the incidence of leukemia-relapse decreases with increasing grades of aGVHD and that cGVHD is associated with a stronger GVL effect than aGVHD (58-123). The best leukemia-free survival was seen in patients with mild acute and chronic GVHD (124). The GVL effect is mediated by donor-derived T-lymphocytes. After HSCT, there is many more donor T-cells than residual host lymphocytes. With less intensive conditioning, a larger amount of host immune cells will survive which can deactivate or destroy some of the donor T-cells, delaying GVHD or its severity. Therefore, donor lymphocyte infusion (DLI) after HSCT is a way to increase the GVL effect (125-126). DLI may induce long-lasting remissions, especially in patients with chronic myeloid leukemia (CML) (127-128). Early disease stage and presence of cGVHD are conditions associated with effectiveness of DLI treatment (129) as is the correlation with development of acute and chronic GVHD after DLI, especially in CML patients (126, 130). To date, almost 300 patients have received DLI at Karolinska, Huddinge.

2.3.2.3 Graft failure/rejection

Graft failure (GF) or rejection is a rare complication that depends on the HLA-matching of the donor, the intensity of conditioning, pre-transplant immunization, or TcD. The frequency of GF is around 1-2% using a HLA-matched sibling donor and 5% using a matched unrelated donor (MUD) (131). For example, for patients with severe aplastic anemia (SAA) immunized by multiple transfusions before the HSCT, the incidence of GF is increased (132); this is also true for patients receiving RIC (leaving enough recipient cells to cause rejection), cord blood graft and TcD graft (61, 133).

2.3.3 Relapse

Relapse remains the most common cause of treatment failure after HSCT in patients with acute leukemia, especially acute lymphocytic leukemia (ALL), compared to other hematological malignancies or diseases. The incidence also depends on the disease stage since the lowest incidence is seen in patients with an early stage of disease, i.e., in first complete remission (CR) or first chronic phase (CP). GVHD decreases the risk of relapse, with the lowest risk for patients with mild aGVHD combined with cGVHD (124). Furthermore, the type of donor is important: using a syngeneic twin donor in transplantations results in a relapse incidence higher than using HLA identical sibling donors (134, 135). TcD of the graft has also been associated with an increased relapse rate probably because of a reduced GVL effect (64, 124, 136). The appearance of leukemia cells below the threshold for standard morphological methods is commonly referred to as minimal residual disease (MRD) (137).

2.3.4 Infectious complications in general

At least one year is necessary to achieve full immune reconstitution of the donor derived immune system post HSCT. Innate immunity including epithelial barriers, monocytes, granulocytes, and NK cells recover within weeks, whereas cells of the adaptive immune system recover much slower. The number of B- and T-lymphocytes may be normalized during the first months after transplantation, but T-cell immunity may remain impaired for years after HSCT. There-
fore, the frequency of infections remains high and is one of the major causes of morbidity and mortality, especially in patients with an unrelated or mismatched donor (45).

During the aplastic or pre-engraftment phase, which occurs 0-30 days after HSCT, damaged mucocutaneous barriers and the lack of functioning immunological cells make the recipients extremely susceptible to bacterial and fungal attacks (138, 139). The oral, gastrointestinal, and skin flora are consequently the sources of infection in this phase. Blood stream infections (bacteremia) due to Gram-positive bacteria such as streptococci species, for example alpha-hemolytic streptococci, are common due to frequent vascular access required for patient care (140-142). Other pathogens that may cause infection are Candida species, coagulase-negative staphylococci, and reactivated Herpes simplex virus (HSV) (143-145). Prophylactic treatments to prevent such infectious complications include decontamination with antibiotics and oral antifungal drugs and isolation routines (146-148). However, patients may be safely treated at home during this phase resulting in less TRM and increased survival (149). The post-engraftment phase spans between day 30 and 100 after HSCT and is dominated by an impaired cell-mediated immunity. The late phase occurs >100 days after HSCT. Both cellular and humoral immunity are still defected during this period. Patients with cGVHD and recipients of graft from MUDs or mismatched donors may especially be at risk for certain infections during this phase (145). Potentially life-threatening infections that mainly occur during the two later phases mentioned include invasive fungal infections, especially aspergillosis and Cytomegalovirus (CMV) infections (4, 150). Invasive aspergillosis is confined to the lungs in the majority of cases, but sinusitis and central nervous system involvement also may occur with some frequency (151, 152). CMV may cause pneumonia, hepatitis, colitis, and super-infections with opportunistic pathogens, in particular in patients with aGVHD (153).

2.4 RESPIRATORY COMPLICATIONS

2.4.1 Pulmonary complications, general aspects

Respiratory complications are frequent in HSCT patients and cause morbidity and mortality in a high proportion of these patients (154-156). The spectrum includes infectious and non-infectious conditions, classified as early or late if the disease occurs within or after 100 days post-transplant, respectively.

2.4.2 Lung defense mechanisms

The lungs are uniquely vulnerable to invasion by noxious agents for two reasons. First, they are exposed to about 10,000 L of inhaled air each 24 hours that contain large amounts of organic and inorganic particles. Second, the lung is the only organ to receive the entire cardiac output so there is a maximum opportunity for circulating agents, such as antigens or immune complexes, to reach the pulmonary bed. Foreign material acting as antigens and able to stimulate antibody formation or participate in immune responses is mainly protein or polysaccharide in nature and generally the larger the molecular weight, the greater immunogenicity. Antigens of special importance in lung disease include bacteria and viruses, plant material including pollen, grains, and molds, mammalian proteins such as serum components and tumor antigens, nucleic acids, and low molecular weight chemicals. Several defense mechanisms protect against these potentially dangerous antigens. The cough reflex consists of irritant receptors in the upper Airways, a cough center in the medulla, a motor output passing to the diaphragm, intercostals, and abdominal muscles. The mucus consists of antimicrobial agents such as immunoglobulins or other...
constituents. Some of these are bactericidal (i.e., lysozyme and interferon), some enhance bacterial adherence to phagocyte cells (complements and interferon), and others protect the airways from proteolytic enzymes (α1-antitrypsin). Impairment of mucociliary clearance is clearly of great importance in predisposing the lung to infection. Cilia activity and tight junctions between epithelial and mucus-secreting cells, creating the epithelial integrity, are also important for defense by preventing excessive transudation of fluid or movement of large molecules. The lower respiratory tract is normally sterile. Organisms reaching the alveoli usually sediment or exit with the next exhalation. Remaining pathogens are normally phagocytosed and killed by the alveolar macrophages (AM) with help of factors such as IgG and complement. In case of infection, the macrophages attract other cells to enhance the defense by liberating several proinflammatory cytokines, recruiting neutrophils and other leukocytes to the infected area. Respiratory infection occurs when the defense mechanisms are overwhelmed or, more commonly, when they fail to function normally. The main factors contributing to development of infection are aspiration of infected upper respiratory secretions (e.g., due to lost or depressed laryngeal or cough reflexes), impairment of removal of secretions (e.g., due to impaired mucociliary clearance and airway obstruction), or impaired immunity, which allows relatively non-pathogenic organisms to become invasive and pathogenic. In particular the two latter options may be heavily impaired after HSCT. Colonization becomes invasive in more severely ill patients (157, 158).

### 2.4.3 Infectious pulmonary diseases

Pneumonias may be caused by multiple pathogens. CMV can be accompanied with bacteria or *Aspergillus* species (spp) by superinfection, and infection with *Aspergillus* spp infection can be accompanied by bacterial agents, CMV, or zygomycetes co-infections. Therefore, assessment should be thorough and cultures or other investigations indicating more than one causing pathogen should not be ignored (4).

![Timeline of infectious pulmonary complications after HSCT.](image)

**Figure 2.** Timeline of infectious pulmonary complications after HSCT.

#### 2.4.3.1 Bacterial pneumonia

Bacterial pneumonias can be defined as a new onset of pulmonary infection with parenchymal alveolar-filling infiltrates in patients with fever if bacteria are found in cultures from blood
and/or sputum. Bacterial pneumonias can also be identified using bronchoalveolar lavage (BAL) cultures, histopathology and/or culture of lung tissue obtained by biopsy or at autopsy, compatible with bacterial pneumonia, or by improvement of clinical symptoms of pneumonia and of infiltrations after institution of empiric antibiotic, but not antymycotic treatment (159). Pneumonias due to bacteria are often seen during the pre-engraftment phase due to the profound neutropenia, but may occur at any time during the post-transplantation period. Mucositis and aspiration due to the influence of opiates and sedatives, containing various bacterial and fungal pathogens, can be predisposing conditions for bacterial pneumonias (160). Pneumonias due to Gram-negative bacteria — such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter, Escherichia coli, Acinetobacter, Moraxella catarrhalis, Chlamydia species, Legionella pneumophila — may occur as well as the intracellular pathogen Mycoplasma pneumoniae (161-166). As in HSCT patients with septicemia, the risk of pneumonia due to Gram-positive bacteria, such as Staphylococcus and Streptococcus species, increases due to the widespread use of indwelling central line (145, 167). More unusual bacteria such as the Gram-positive rods Noocardia asteroides and Actinomyces may cause pneumonia in severely immunosuppressed patients (168).

Encapsulated bacterial infections, such as Haemophilus influenzae (HI) and Streptococcus pneumonia, are more common during the late phase (> 100 days post HSCT) (145, 159). Infections with Mycobacterium tuberculosis and non-tuberculous mycobacteria (NTM) — for example, Mycobacterium avium intracellulare — are extremely uncommon causes in non-endemic countries (169-172). However, in endemic countries, the risk of tuberculosis is not neglectable. Risk factors for tuberculosis are allogeneic HSCT, TBI and cGVHD (170, 173).

2.4.3.2 Protozoa

Toxoplasma gondi is a rare cause of pneumonia in allogeneic HSCT patients. The presence of this parasitic protozoa mostly occurs within the first six months after HSCT as a result of reactivation in patients who are seropositive for toxoplasmosis pre-transplant (174, 175).

2.4.3.3 Fungal pneumonia

In 2002, the definitions of invasive fungal diseases (IFD) were revised to distinguish dubious cases from the more likely ones, since the originally published definitions allowed too many dubious cases to be included. After the revision, IFD were categorized as proven, probable, or possible. “Proven” IFD requires histological analysis or culture from a tissue taken from the site of disease. In contrast, “probable” IFD requires the presence of a host factor and clinical and mycological criteria consistent with IFD. In cases with “possible” IFD, the same criteria as probable IFD are met, except that mycological criteria are absent (176). However, the failure to meet the criteria for IFD does not mean that there is no IFD, only that there is insufficient evidence to support this diagnosis.

Pneumocystis jiroveci was previously described as a protozoa, but has via DNA-analysis shown to be a fungus (177). This pathogen was not included in the revised definitions of IFD. Radiological findings are usually bilateral interstitial-alveolar infiltrates, but normal radiographs may also be found (178). The relationship between decreased CD4 positive T-cell counts is not as well established after HSCT as it is in patients with acquired immunodeficiency syndrome (AIDS). Instead, corticosteroid therapy, especially in patients with cGVHD seems to be a risk factor for development of pneumocystis pneumonia (PCP) (179, 180). Pneumonia due to
Pneumocystis jiroveci has previously usually occurred one to five months post-transplant (181). However, due to the widespread use of prophylactic co-trimoxazole or pentamidine treatment, PCP has become a rare complication that usually occurs late after HSCT (181, 182).

Aspergillus is the most common fungus associated with pneumonia and has been reported in 10 to 15% in HSCT recipients (183, 184). The risk factors include prolonged neutropenia, broad-spectrum antibiotics, disruption of mucosa, GVHD and treatment with corticosteroids (154). However, there has been a notable increase in the frequency of post-engraftment Aspergillus infections, while at the same time there has been a decrease in risk during the pre-engraftment phase, probably partly due to the shortened period of neutropenia due to the use of PBSC and better prophylaxis (183-185). Establishing a definitive diagnosis is still a challenge, since in previous reports up to 30% of the cases remain undiagnosed pre-mortem (186). Radiographic investigations may show nodules, cavities, and/or consolidation. The “air crescent sign” seen on computer tomography’s (CT) is a nodular soft tissue opacity surrounded by a crescent of air, which is a recent finding. Another characteristic finding is the “halo sign”: nodular opacity surrounded by a rim (i.e., halo) of ground glass attenuation representing edema or hemorrhage (187).

Candida species are frequently asymptomatic colonizers of the upper respiratory tract. Ideally, hematogenous Candida pneumonia related to invasive candidiasis should be distinguished from isolated lung infection. However, in the clinical practice such discrimination is very difficult. Therefore, some authors have previously suggested the concepts “primary Candida pneumonia” (invasive infections limited to the lungs) (188) and “secondary Candida pneumonia” (lung involvement due to hematogenous dissemination) (189), which occur in patients with reduced or altered host defenses. Primary Candida pneumonia is most often due to Candida albicans. Non-albicans spp implicated in primary forms are Candida tropicalis and Candida parapsilosis. The species distribution of secondary pneumonia is Candida albicans, tropicalis, glabrata, parapsilosis, as well as krusei (190, 191). Candida pneumonias are exceedingly difficult to diagnose ante-mortem. Cultures from sputum or bronchoscopy samples are booth poor predictors of tissue invasion (192) and many of the patients may have normal radiographic findings. However, if chest radiographs are abnormal, they may show bilateral interstitial or alveolar patchy infiltrates and sometimes cavities and cysts (189, 193, 194).

The zygomycetes, including Rhizopus and Mucor cause IFD with a prevalence of 1.9% (195, 196). However, when they cause disease in HSCT patients, the lungs are the most common site of infection with a reported mortality rate of 60-80%. Risk factors include severe neutropenia and corticosteroid treatment for aGVHD or cGVHD (197). Aspergillus and zygomycetes may cause extensive hemorrhaging due to their angioinvasive tendency (151).

2.4.3.4 Viral pneumonia

Pneumonia due to CMV has previously most often occurred within the first 100 days after HSCT (154). However, the use of prophylactic strategies have both reduced the incidence and postponed the onset of disease (198, 199). Patients who develop cGVHD seem to be especially prone to develop late CMV pneumonia (200). High-resolution CT (HRCT) may show bilateral, diffuse, or patchy ground glass opacities (201, 202). One prophylactic approach is to give all patients at high risk for CMV antiviral prophylaxis during a defined period post-transplantation. Another strategy is to use preemptive therapy, where antiviral therapy is given in case of sub-
clinical viremia detected by the highly sensitive pp65 antigenemia or by polymerase chain reaction (PCR) assay (148, 203-205). In the majority of the cases, CMV pneumonia is a result of reactivation of latent endogenous virus due to immunosuppression. In some of the seronegative recipients, CMV is caused by infection from seropositive donor-graft or transfusion of blood products from seropositive donors (206). Previous reports have shown an increased mortality in CMV-seropositive patients receiving marrow from seronegative donors and a lower CMV-related mortality in seronegative patients receiving seronegative HSC transplant (207-208). Seronegative recipients of seropositive graft have a lower risk of CMV disease after HSCT than do seropositive recipients (209). Today all blood products are filtered to remove leukocytes to minimize this risk, but there is still a mortality disadvantage for the seropositive patients (210). The mortality rate of CMV pneumonia has historically been as high as 85% of the transplant recipients (151). Today, as a result of better prophylactic strategies and routines, CMV disease during the first three months post-transplantation has been reduced. However, CMV pneumonia is still the most serious manifestation of CMV in HSCT recipients (211).

Because oral administration of acyclovir is an effective prophylaxis (160), pneumonia due to HSV and the Herpes zoster virus (HZV) is rare. Most patients with HSV pneumonitis have mucocutaneous involvement and pneumonia occurs by contiguous spread from oropharynx to the trachea. HZV occurs in the setting of disseminated infection and viremia (151, 212). HRCTs may show areas of focal or multifocal ground-glass opacities or diffuse pneumonia when viremia occurs (154, 213).

Some retrospective studies have shown that reactivation of Human herpes virus-6 (HHV-6) is higher with the use of cord blood than bone marrow or PBSC (214). HHV-6, which causes roseola in children, has been found in lung tissues from HSCT patients with pneumonia, suggesting a pathogenic role and therefore is considered to be a potential cause of interstitial pneumonitis (215). Reactivation of HHV-6 has also been associated with the occurrence of GVHD, Epstein-Barr virus (EBV) coinfection and unrelated donor transplantation (216). Respiratory syncytial virus (RSV) is a commonly isolated viral pathogen in HSCT patients (217). The risk of RSV pneumonia is much higher during the pre-engraftment phase than after one month post-HSCT (218). Since the mortality is high once pneumonia develops, antiviral treatment should be given immediately at the onset of upper respiratory illness (218). Other causes of pneumonitis are Parainfluenza virus and Influenza A and B. Mortality due to these agents are lower than for RSV but may be associated with high morbidity (154, 219). Adenovirus, which is the most common viral pathogen in children but uncommon as the cause of pneumonitis, is responsible for significant mortality post-HSCT (220, 221). The increased risk of Adenovirus infection may be correlated with the lack of endogenous T-cell immunity (222). Another serious complication with mostly fatal outcome is reactivation or infection with Epstein-Barr virus post-HSCT, which most often induces post-transplant lymphoproliferative disease (PTLD) (223). Even if EBV-associated PTLD accompanied with EBV-associated pneumonia is rare, several case reports describe this condition with rapidly progressing dyspnoe, hyperpyrexia, and multifocal, patchy and diffuse, bilateral ground-glass infiltrations on chest CTs (224-226). However, the development of EBV specific T-cells may cure severe cases of PTLD (227).

### 2.4.4 Non-infectious pulmonary complications
Respiratory disorders of non-infectious character have during the past years become the major cause of pulmonary morbidity and mortality in HSCT patients, since effective prophylactic strategies and treatments against different pathogens have been introduced that have decreased the incidence of infectious complications (155, 156, 228).

**Figure 3.** The chronology of non-infectious pulmonary complications after HSCT.

### 2.4.4.1 Early non-infectious complications

Pulmonary edema is the most common complication and usually occurs the second or third week post-transplantation. Chest radiographic findings cannot be distinguished from pulmonary edema of other etiology. The increased capillary permeability and/or hydrostatic pressure are probably a result of the large volumes of intravenous transfusions of blood, nutrition, hydration, and toxicity due to the conditioning regimens. Non-cardiogenic pulmonary edema can also be due to lung injury caused by immunosuppressive medications, sepsis, and aspiration (151, 229).

Diffuse alveolar hemorrhage (DAH) may develop in HSCT recipients (155) and is manifested by widespread, rapidly progressing alveolar injury with diffuse multilobar infiltrates, and symptoms and signs of pneumonia although absence of infection or another etiology. The syndrome usually occurs two to three weeks after HSCT (230) and the ventilator defect is restrictive (155, 231). BAL with a progressively bloodier return in at least three lobes is a hallmark of the syndrome. However, platelet counts are not lower than in patients without DAH (232) and hemoptyses are rare (231). According to previous reports, risk factors are age > 40 years, TBI, transplantation for solid tumors, high fever, severe mucositis, and renal insufficiency (151). DAH is associated with high mortality (80-100% with only supportive care) but an early diagnosis and administration of high dose steroids improves the result (155, 233). Although the exact cause of DAH is unknown, DAH as well as idiopathic pneumonia syndrome (IPS) is believed to emerge from acute lung injury induced by the conditioning strategies with chemotherapy, radiation, and perhaps occult infection. Many cases occur at the time of engraftment with neutrophil influx into the lungs, which may accentuate the injury (151, 229).
Idiopathic pneumonia syndrome accounts for nearly 50% of all the interstitial pneumonitis after HSCT (234). The reported incidence of IPS is about 10%, ranging between 2-17% (155). However, in one study the incidence of IPS in allogeneic HSCT recipients was significantly lower with nonmyeloablative than with myeloablative conditioning regimens (2.2% versus 8.4%) (235). IPS is defined as a diffuse lung injury after BMT/HSCT for which an infectious or non-infectious etiology is not identifiable (156, 236). Instead, the etiology is thought to be multifactorial, encompassing damage sustained from conditioning related to toxic regimes and immunological dysregulation (237). BAL, transbronchial biopsy (TBB), or open lung biopsy (only performed if the condition of the patient permits) may establish the diagnosis and exclude infectious etiology. The two main histological patterns are interstitial pneumonitis and diffuse alveolar damage (229, 236). As in DAH, the chest radiographs may show diffuse multilobular infiltrates, and the symptoms are the same as for pneumonia — dyspnoea, fever, non-productive cough, and increasing hypoxemia. The median time of onset is 42-49 days, but there is also an early peak 14 days after HSCT (236, 238). Late forms have also been described (237). Pulmonary physiology tests are abnormal with an increased alveolar to arterial oxygen gradient and restrictive lung function. In the majority of patients, the course is rapid and the mortality is around 74% (151). IPS may be complicated by superimposed infections, pneumomediastinum, and subcutaneous emphysema (155, 239). However, the clinical spectrum is broad and in about 30% the pneumonia resolves (155, 238). Risk factors for development of IPS are higher age, high intensity conditioning, transplantation for a malignancy other than leukemia, and high grade acute GVHD (155, 235, 238). There is no specific treatment that has been proven effective for IPS, not even high dose corticosteroids (155, 238, 240). However, a few reports have noted that Etanercept, a soluble TNF-α-binding protein, is associated with improvement of the lung function and decreased lung injury (240, 241). Another novel strategy may according to recent reports be blocking of the receptor-ligand interactions between chemokine receptor 2 (CCR2) and its primary ligand monocyte chemoattractant protein-1 (MCP-1) (242).

Radiation pneumonitis is mostly an asymptomatic condition with only radiographic changes. Only 7% of the patients have symptoms such as cough and dyspnoea. Pulmonary function tests (PFTs) reveal restrictive lung physiology and reduced diffusing capacity. A reduction of the diffusing capacity is the best way to predict pulmonary damage due to radiation. Corticosteroids are the preferred treatment (229). The mortality is high in patients with an early onset of symptoms (243) and in them who progress to respiratory failure before corticosteroid treatment.

Acute GVHD develops in several patients after HSCT, but pulmonary complications due to aGVHD are minimal (229).

Pulmonary veno-occlusive disease is a rare complication, occurring six to eight weeks after HSCT, and is dominated by progressive dyspnoea, hypoxemia, radiographic signs of pulmonary edema, and signs of pulmonary hypertension. Since this condition also has been reported in non-transplanted patients who have received chemotherapy and radiation and in patients with viral infections, it has been suggested that this complication is a result of an infectious or toxic injury to the endothelium (155). Multiple HSCTs have also been implicated as an etiological factor (229).

Bronchiolitis obliterans organizing pneumonia (BOOP), also known as cryptogenic organizing pneumonia (COP), is a less common early complication that occurs in < 2% of allogeneic
HSCT recipients. It is important to differentiate this diagnosis from bronchiolitis obliterans (BO), since it has different clinical and pathological features and outcomes. Histologically, BOOP is characterized by granular plugs of bronchioles that, unlike BO, extend into the alveoli. Unlike BO, BOOP usually occurs during the first 100 days after HSCT and the PFTs show a restrictive, not as in BO obstructive, ventilation defect (155). Diffusing capacity of the lung for carbon monoxide (DLCO) is usually reduced in contrast to BO patients who may have a normal DLCO. Furthermore, the clinical presentation differs from BO with an acute onset of dyspnœa, cough and fever, whereas BO is insidious and with no appearance of fever. The radiological findings are also different and may show patchy and usually peripheral consolidations, ground glass attenuation, and nodular opacities. In contrast to BO, which is an irreversible process, BOOP is potentially reversible with a good response to treatment with corticosteroids (156, 244).

2.4.4.2 Late non-infectious complications

Bronchiolitis obliterans is the most common late non-infectious pulmonary complication after HSCT and is characterized by a new onset of air flow obstruction (AFO) after HSCT (151, 244). The reported incidence varies widely and ranges in different studies between 0-48% (244). This variation depends on the previous lack of uniform BO criteria. However, recently the National Institutes of Health (NIH) contributed to a consensus document that identified clinical, spirometric, and radiological criteria for BO (245). The majority of cases occur between six and twelve months after HSCT, although there are also reports of BO as early as 30 days post-transplantation (244) or later than one year post-transplantation (246). The clinical course is variable. In a majority of patients, there is a slowly progressing AFO with episodes of acute exacerbation. Only a minority of the patients has a rapidly progressing disease with development of respiratory failure. Approximately 20% of the patients are asymptomatic despite abnormal lung function tests and in the early stages chest radiographs may be normal. However, in later stages HRCT often shows bronchiectasies, signs of expiratory air trapping, and a mosaic pattern due to hypo attenuation alternating with areas of ground glass appearance (247). The etiology is unknown. Proposed mechanisms are lung injury due to the conditioning regimens, infectious processes (especially viral infections), recurrent micro-aspirations due to esophagitis related to chronic GVHD, or an alloreactive immune process in which the bronchial epithelium of the recipient may be the target of the donor’s CTLs (155). The inflammatory cytokines involved in development of BO include IL-1, IL-6, IL-8, IL-18, and TNF-α (248-250). Even if the effect of therapy is mostly poor, aggressive therapy may in some patients improve or stabilize the lung function (251, 252). The prognosis has been reported worse in patients who do not respond to the treatment, in patients with progressive cGVHD or who have a history of viral infection, or in patients who have a rapid decline of FEV1 or who develop airflow obstruction early after HSCT (253- 256). The mortality rates vary widely with a range of 14-100% with an overall mortality of 65% three years after transplantation (155, 244).

Chronic GVHD is the main risk factor for BO. Pulmonary involvement is common in patients developing cGVHD and median time of onset of respiratory symptoms five months (range 1-13 months) (257). Previous studies suggested that BO can not develop without the appearance of cGVHD (251, 258); however, recent studies from large HSCT centers report that up to 7% of the patients develop BO without coincident cGVHD (256).
Radiation fibrosis is another late non-infectious complication. Chest radiography, including CTs, is indistinguishable from pulmonary fibrosis of any other etiology and the severity ranges from asymptomatic to respiratory failure. The most common risk factor is previous radiation pneumonitis (243). Pneumothorax may also occur in these patients, especially patients who have end stage lung disease (229).
3 AIMS OF THE PRESENT STUDY

Both infectious and non-infectious pulmonary complications remain one of the most common causes of mortality and morbidity after allogeneic HSCT. This project investigates the circumstances and risk factors that may lead to the development of respiratory disorders due to transplantation. In addition, this study evaluates current diagnostic methods to detect these kinds of complications at an early stage. The specific aims of the four studies that comprise this project are listed below:

Study 1. To evaluate the incidence, outcome, etiology and risk factors for death due to the infectious complications associated with pneumonia within 100 days after HSCT (early pneumonia) over three decades at Karolinska University Hospital, Huddinge.

Study 2. To determine the use of and possible disadvantages with BAL and the value of cultures from other specimens as a diagnostic tool for pneumonia.

Study 3. To determine the incidence and risk factors for the non-infectious complication bronchiolitis obliterans at our center and to evaluate the use of radiology and spirometry to establish the diagnosis and to study the course and outcome for this patient cohort.

Study 4. To study the incidence, risk factors, and etiology of death due to early pneumonia and the overall pneumonia-related death in patients receiving a reduced intensity-conditioning regimen (i.e., non-myeloablative conditioning) compared to patients receiving a myeloablative-conditioning regimen.
4 MATERIALS AND METHODS

4.1 PATIENTS AND DONORS

All patients in these studies underwent allogeneic HSCT at Karolinska University Hospital (Huddinge, Sweden) between 1997 and 2009. The participants gave verbal and written consent and the Karolinska Institute ethics committee approved the studies (DNR 425/97 and 2011/972-31/1). Most of the patients received HSCs from HLA-A, HLA-B, and DR-identical sibling donors. However, since 1990, the number of unrelated donors has increased (Figure 4). Patient and donor characteristics in Study I-IV are presented in Table 1a.

Figure 4. The number of related versus unrelated donors in allogeneic HSCT 1975-2009, Karolinska University Hospital, Huddinge
Study | I | II | III | IV
--- | --- | --- | --- | ---
**Number of patients** | 997 | 450 | 527 | 691
**Males/Females** | 586/411 | 262/188 | 295/232 | 413/278
**Age (Median/Range)** | 29 (<1-77) | 37 (<1-77) | 34 (<1-77) | 38 (<1-77)
**Diagnosis:**
Acute leukemia | 469 | 192 | 242 | 288
Chronic leukemia | 209 | 71 | 110 | 84
MDS/MPS | 49 | 36 | 34 | 78
Lymphoma | 39 | 31 | 26 | 49
Solid tumor | 38 | 50 | 38 | 60
Other hematological malignancy | 64 | 19 | 21 | 32
Non-malignant disorder | 129 | 51 | 56 | 100
**Disease stage (Early/Late)** | 547/412 | 206/194 | 260/229 | 316/315
**Donors:**
HLA-identical related | 579 | 180 | 223 | 259
Twin | 11 | 4 | 4 | 4
MUD | 302 | 206 | 246 | 325
Mismatched unrelated/related | Tot 105 | Tot 60 | Tot 54 | 97/6
**Stem cell source** | 743/249/5 | 163/275/12 | 280/247/0 | 186/451/54
BM/PBSC/CB
**NC dose (×10⁸) (Median/Range)** | 2.9 | 7.5 | 4.6 | 9.1
(0.03-80) | (0.14-80) | (0.03-80) | (0.014-86.3)
**CD 34 dose (×10⁶)** | 5.8 | 6.8 | 5.8 | 6.9
(0.1-56.4) | (0.1-66.0) | (0.1-56.4) | (0.03-80)
**Female donor to male recipient** | 242 | 80 | 101 | 129
**G-CSF after HSCT** | 356 | 244 | 341 | 19
**Previous HSCT (auto/allo)** | 43 | 63 | 42 | 69/32

Table 1a. Patient and donor characteristics in Study I-IV.

### 4.2 CONDITIONING PROTOCOLS

The conditioning protocols have differed somewhat during the study years depending on diagnoses and time of study. The alternative therapies were TBI-based conditioning, non-TBI-based, or RIC regimens. The regimens used in Study I-IV are presented in Table 1b.

TBI-based conditioning, the most commonly used regimen, consisted of Cy (60 mg/kg) i.v administered for two consecutive days before HSCT (either days -5 and -4 or -4 and -3) and TBI single dose (7.5-10 Gy) on day -1, with the lungs shielded to receive no more than 7-9 Gy (259). Cy (120 mg/kg) and TBI (7.5 Gy) (7.0 Gy to the lungs) were given to patients who received a TcD graft (260). They were also given 2 Gy of total lymph node (TLI) irradiation on days -8, -7 and -6. Previously, TBI (10 Gy) was given during one session, but now fractionated...
3 Gy of TBI (fTBI) is administered for four consecutive days to decrease radiation side effects such as pneumonitis (261).

Non-TBI based conditioning consisted mainly of Bu (4 mg/kg/day for four consecutive days from day -8 to -5) combined with Cy (120 mg/kg/day), a treatment strategy that is known as the BUCY regimen (262). Patients with metabolic disorders received Bu (80 mg/m²/day) for four days followed by Cy (2 g/m²/day) for four more days (263). Patients with SAA (with an HLA-identical sibling donor) were given Cy (50 mg/kg) for four consecutive days (264). Until 1996, all patients received MTX (12 mg) intrathecally twice before HSCT. This regimen is now only used for patients with a history of central nervous system (CNS) leukemia. In addition to BUCY, some patients received Vepecide (VP) (300 mg/m²) or Melfalan (140 mg/m²). Other combinations of chemotherapy have also been used.

ATG (2-5 mg/kg/day) for two to five days, Campath (30-100 mg) or OKT-3 (5 mg/day) for up to five days, was administered to patients with unrelated or mismatched donors and to patients with non-malignant diseases independent of donor (65).

Reduced intensity conditioning was mainly given according to five protocols (48, 265):
1) FLU 150-180 mg/m² and Cy 60-120 mg/kg;
2) FLU 150 mg/m² and treosulfan 42 g/m²;
3) FLU 180 mg/m² and Bu 8 mg/kg;
4) FLU 150 mg/m² days and 2 Gy of TBI; and
5) FLU 90 mg/m², Cy 120 mg/kg and 2 × 3 Gy fTBI.

4.3 GVHD PROTOCOLS

GVHD prophylaxis

Various protocols to prevent GVHD have been used. From 1975 to September 1985, MTX was given as a monotherapy (266). Between 1982 and September 1985, CsA was used as a monotherapy during the first year after HSCT (267). Between October 1985 and October 1989, adult leukemia-patients were included in a randomized study with either MTX in combination with CsA or TcD (260). All other patients received a short course of MTX (15 mg/m²) (on days +1, +3, +6, and +11) and CsA daily (56). TcD was also given to patients with unrelated HLA-mismatched donors (268). Between 1989 and 1993, the prophylaxis was individualized so that patients at high risk for GVHD received CsA in combination with MTX, and patients at low risk received MTX alone. This strategy was done due to reports of lower relapse risk without CsA (269). However, since 1993 MTX together with low dose CsA (the concentration of CsA kept at 100 ng/ml) has been the regimen of choice for patients with hematological malignancies and HLA identical sibling donors (54, 270). Recipients of unrelated grafts have received higher doses of CsA in combination with four doses of MTX (271). Patients at high risk of liver toxicity, and those receiving a cord blood graft, received prednisolone combined with CsA rather than MTX. The prophylaxis was gradually tapered from two months onwards for patients with malignant diseases without GVHD. Patients given non-myeloablative conditioning received CsA + MTX, CsA alone, or CsA + MMF (15 mg/kg day 0 to +45) (272). Other combinations used most recently are tacrolimus in combination with MTX, MMF, or sirolimus (273-275). The GVHD prophylaxis regimens used in Study I-IV are presented in Table 1b.
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<th>III</th>
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Table 1b. Conditioning and GVHD prophylaxis regimens in paper I-IV.

GVHD treatment
The diagnosis of both aGVHD and cGVHD was established by clinical symptoms and/or biopsies of affected organs. Prednisolone (2 mg/kg/day for adults or 2-3 mg/kg/day for children) for at least one week was immediately instituted in case of signs of aGVHD grade I, and then, if possible, the dose was tapered (276). In more advanced cases of aGVHD, methylprednisolone (i.v), ATG, MTX 5-10 mg/m² once a week and/or PUVA, was administered (277). Treatment modalities of cGVHD included prednisolone, CsA, azathioprine, thalidomide, TLI, and/or PU-VA or extracorporeal PUVA (146). Recently, MMF, antibodies against TNFα, IL-2 receptor antagonists, and MSCs has also been used to treat manifest aGVHD or cGVHD (278, 279).

4.4 SUPPORTIVE CARE
Isolation routines. Before 1997, patients were isolated at the hospital from the start of Cy until the ANC reached > 0.5 x 10⁹/L. Since January 1998 ANC > 0.2 x 10⁹ for two consecutive days was the limit to end the isolation. From September 1997, patients were freed from isolation during weekends and evenings and since March 1998, they have been offered home care (280).
Transfusion routines. Platelet and blood products were irradiated with 15-20 Gy and filtered before administration. Transfusion of platelets was administered when the counts were < 30 × 10^9/L and erythrocyte transfusions when the hemoglobin level was < 70 g/L. Granulocytes were administered only to patients with life threatening systemic infection, high fever, and severe mucositis or local infections not responding to antibiotics.

Growth factors. According to different study protocols, G-CSF 5 (µg/kg) was given from March 1993 until December 1997 (281) and from January 1998 to all patients from day +10 to September 2001. However, in September 2001 the regular administration was stopped due to reports of a G-CSF associated increased GVHD incidence (282).

Prophylactic routines to prevent infections. Between 1980 and 1985, a combination of polymyxin B, nalidixic acid, and oral amphotericin B was given to patients > 12 years of age from the start of conditioning until engraftment as prophylactic gut decontamination. Between 1986 and 1993, rather than the above combination of drugs, ciprofloxacin (500 mg twice daily) was combined with oral amphotericin B (283). Children < 12 years of age did not receive this kind of therapy. From 1985, patients with HSV IgG titers ≥ 10,000 /L or a previously recurrent HSV infection received prophylaxis with acyclovir (200 mg x 4 daily) during the aplastic phase (284). In addition, co-trimoxazole was administered for six months or as long as cGVHD was present after HSCT, to avoid infection with Pneumocystis jirovecii (4).

Empirical and pre-emptive therapy. Broad-spectrum antibiotics, such as erimethoprim / sulphametoxazole (i.v) in combination with aminoglycoside, were given immediately after gathering of two bottle cultures of blood, if the body temperature was above 38.5°C or 38°C at least twice within 24 h. Since 2005 instead meropenem is the preferred first line antibiotic therapy. In case of four or five days of high fever despite antibiotics, indicating possible fungal infection, Amphotericin B (i.v) was added (283). Viral monitoring of CMV with preemptive therapy has been used since 1990. Between 1988 and 1991, a rapid isolation technique was applied (209). Since 1991, weekly blood samples were gathered from CMV-seropositive patients or CMV-seronegative patients with seropositive donors screening for CMV-DNA in leukocytes, a PCR-based technique (146, 285). Between 1991 and 2002, > 10 copies of CMV-DNA per 200,000 peripheral blood leukocytes (PBL) in two consecutive samples was enough to start preemptive CMV therapy, but since 2002, due to study results, > 100 copies is the basis for starting this kind of therapy, consisting of either ganciclovir (5 mg/kg twice daily) or foscarnet (90 mg/kg twice daily) for two weeks (146). If the test turned negative, the therapy was ended after two weeks, otherwise it continued but only once a day. Patients with CMV pneumonia received ganciclovir in combination with immunoglobulin i.v (286).

4.5 STATISTICAL ANALYSES

Study I, III and IV. The cumulative incidence rates of BO or death from pneumonia were established using an estimator of cumulative incidence curves taking competing events into consideration and compared using Gray’s test (287). Competing event was death without BO or death not due to pneumonia. To establish the risk factors for BO or death from pneumonia univariate and multivariate risk factor analyses were performed using the proportional subdistribution hazard regression model developed by Fine and Gray. Factors ≤ 10% level were extracted from the univariate analyses and then introduced to the stepwise elimination multivariate analyses. The effect of cGVHD and DLI on the incidence of BO was also analyzed as time dependent varia-
bles by performing multivariate analyses with Cox proportional hazard model including time dependent variables. All tests were two-sided. The type I error rate was fixed at 0.05 for factors potentially associated with time-to-event outcomes. Categorical parameters were compared using the Chi-square test and continuous variables were compared using the Mann-Whitney U-test. Analyses were performed using the cmprsk package (developed by Gray, June 2001), Splus software version 6.2 (Insightful, Seattle, WA, USA), and Statistica software (StatSoft, Tulsa, OK, USA).

4.6 FLEXIBLE FIBEROPTIC BRONCHOSCOPY AND BRONCHOALVEOLAR LAVAGE

In most patients, flexible fiber optic bronchoscopy (FFB) was performed under local anesthesia in case of persistent respiratory symptoms and infiltrations on chest radiographs despite antibiotic and/or antifungal therapy for three to four days, if their performance status allowed this procedure. Patients with a platelet count below 50 x 10⁹/L were given 200-250 ml platelet infusion starting no more than one hour before the bronchoscopy. In addition, the patients were not allowed to eat or drink for at least six hours before the procedure, and were premedicated with morphine, ketamine, or midazolam i.v and atropine subcutaneously. Lidoicaine hydrochloride was administered topically into the mouth and pharynx and then as needed in the bronchial tree during the examination. Monitoring of blood pressure, heart rate variability, and oxygen level was done and external oxygen supplied if needed. The bronchoscope was inserted to the mouth, nose, or through an endotracheal tube in a few mechanically ventilated patients. Following inspection of the bronchial tree, BAL was performed by wedging the bronchoscope into the bronchial segment showing the worst radiographic appearance or into the right middle lobe if the disease was diffuse. A volume of 120-150 ml of normal saline was lavaged in 25 to 50 ml aliquots, aspirated separately by vacuum suctioning, and then pooled (288-290). Bronchial washing was performed by instillation and aspiration of 2-4 ml saline in a suitable segment and bronchial brushing mostly with a protected, double-sheathed brush (BFW brush) under visual control (288). In the majority of patients with pneumonia, TBBs were avoided due to the risk of bleeding and pneumothorax and in BO patients also due to the most often low diagnostic value (291, 292).

4.7 PULMONARY FUNCTION TEST

Spirometry, measuring the speed (flow) and/or the amount (volume) of air that can be inhaled or exhaled, is the most common pulmonary function test. PFTs were performed in most allogeneic HSCT patients before transplantation, but not regularly after HSCT unless the patient had symptoms or signs of respiratory dysfunction.

Dynamic spirometry. The most common parameters are vital capacity (VC), forced vital capacity (FVC); forced expiratory volume in one second (sec) (FEV₁), forced expiratory flow (FEF) (25-75%), and maximal volume ventilation (MVV). FVC is the volume of air that can forcibly be exhaled after full inspiration and is measured in liters. FVC may be decreased, for example, by premature closure of airways in expiration. FEV₁ values of 80-100% of the average value are considered normal. FEV₁ may be diminished if there is an increased airway resistance to expiratory flow. In healthy adults, FEV₁/FVC should be approximately 75-80% but is decreased in patients with an obstructive disease. In restrictive diseases, such as pulmonary fibrosis, the FEV₁ and FVC are both reduced proportionally and the value may be normal or even increased.
as a result of decreased lung compliance. Recent research suggests that the prevalence and onset of a decline of FEF of 25%, 50% or 75% may be even more sensitive than FEV₁ in order to detect small airway obstructive disease (293, 294). FEF is the flow or speed of air coming out of the lung during the middle portion of a forced expiration. The value can be given at discrete times, which means what fraction remains of FVC. The usual intervals are 25%, 50%, and 75% (FEF₂₅, FEF₅₀ and FEF₇₅) of FVC. It can also be given as a mean of the flow during an interval, that is generally delimited by when specific fractions remain of FVC, usually 25-75% (FEF₂₅₋₇₅). Values ranging from 50-60% and up to 130% of the average values in a healthy population are considered normal. MEF is maximal (mid-) expiratory flow. This parameter is theoretically the same as peak expiratory flow (PEF), although the first mentioned parameter is measured in L/second and PEF values in L/minute. The volume and flow parameters are shown in Figure 5.

![Flow (L/Sec)](Image)

**Figure 5.** Dynamic spirometry, volume and flow parameters.

**Static spirometry.** Lung volume and lung capacities (the latter inferred from the lung volumes) refer to the volume of air that is associated with different phases in the respiratory cycle. Important lung volume parameters are tidal volume (TV) and total lung capacity (TLC). TV is the volume of air inspired or inhaled in a single breath at rest, most often around 500 ml or 7 ml/kg body weight. TLC is the maximum volume of air present in the lungs. The average value in an adult human male is about 6 L. The expiratory residual volume (ERV) can be measured by a spirometer, but the residual volume (RV) and the residual functional capacity (FRC) must be measured by other methods, such as a plethysmograph. FRC is the volume of air at the end of a passive expiration, which is the sum of ERV and RV. The test is done by enclosing the patient in a plethysmograph or a body box and measuring mouth pressure and body box pressure changes. The lung volumes and capacities are shown in Figure 6.
Figure 6. Static spirometry, lung volume and lung capacity values.

Diffusing lung capacity of carbon monoxide is the carbon monoxide (CO) uptake from a single inspiration in a standard time, which is usually 10 sec, and measures the lungs’ ability to transfer gases. While oxygen uptake may be limited by diffusion in circumstances of low ambient oxygen or high pulmonary blood flow, CO2 is not limited by diffusion under most circumstances. To measure DLCO the patient inhales a test gas mixture (a full VC inhalation) that contains a small amount of CO (usually 0.3%) and some helium or other non-absorbed tracer gas, holds his/her breath for ten seconds, and rapidly exhales. The first part of the expired gas is discarded, but the next portion, which represents gas from the alveoli, will be collected. By analyzing the concentration of CO and helium in the inhaled versus the exhaled gas, calculation of how much CO was absorbed can be performed. This method is known as the single breath diffusing capacity test (295). Diffusing impairments associated with thickening and/or damages of the alveolar walls may be picked up in this way, in patients e.g., who have lung fibrosis or emphysema. However, DLCO must be corrected in case of anemia or pulmonary hemorrhage (296). Low concentration of hemoglobin in the erythrocytes can reduce DLCO and pulmonary hemorrhage can artificially increase the value due to an excess of erythrocytes in the interstitium or the alveoli.
5 RESULTS AND DISCUSSION

5.1 RISK FACTORS FOR DEATH DUE TO EARLY PNEUMONIA (STUDY I)

In the first study, we retrospectively studied causes of early death (within three months after HSCT) in 997 allogeneic HSCT patients over three decades. Although HSCT is the only curative treatment for some hematological malignancies, success is limited by TRM. Previous studies have identified pulmonary complications as one of the remaining most important causes of morbidity and mortality after HSCT (93, 138, 297). These kinds of complications include various non-infectious and infectious complications such as pneumonia. We aimed to study whether there was a change over time of the incidence, etiology, and risk factors for death due to early pneumonia and if so, what the main reasons may have been. We found that pneumonia had been the most common cause of death in the 144 patients who died within three months after the HSCT with a cumulative incidence of 5.7% compared to 10% for all other early-death causes (Table 2). We observed striking changes. The cumulative incidence of early death from pneumonia was 8.9% for the first decade studied (1975-1985), 8.2% for the second decade (1986-1995), and 2.8% for the third decade (1996-2003). In the univariate analysis, eleven risk factors were found to be possibly related to early pneumonia death (p≤0.10) and these risk factors were introduced to the stepwise eliminating multivariate analysis. In the multivariate analysis, however, only three of these eleven factors were significantly associated to early pneumonia death: receiving a TcD graft (p<0.001), the year of transplantation (p<0.001), and bacteremia (p<0.001) (Figure 7).

For a few years during the 1980s and the early 1990s at our center, TcD was administered mainly to patients with a mismatched donor or included in a randomized study (260). In the early 1990s, however, this method was abandoned due to reports of increased incidences of relapse, graft rejection, and infections (34, 61-63). In previous studies, a prolonged and profound T-cell immunity was found especially in adult patients receiving an unrelated TcD graft, which may have been one of the reasons for the increased risk of pneumonia (198). In our study, the cumulative incidences of death from pneumonia with and without TcD were in patients with a sibling donor 21.9% and 5.4%, respectively and with a mismatched donor 18.2% and 8.1%, respectively, indicating that TcD was superior as a risk factor compared to HLA-match. Furthermore, bacteremia was identified as a risk factor. Bacteremia, defined as the first positive blood culture related to a febrile episode, is a well-known common complication during the neutropenic phase and contributes frequently to the development of bacterial pneumonia (160, 283). In our study, based on results from clinics, chest X-rays, cultures, and tissue samples from autopsy alone (n=5), autopsy and bronchoscopy (n=4), and bronchoscopy alone (n=2), ten of the patients were considered to have died due to Gram-positive bacteria (n=7) or Gram-negative bacteria (n=3). Another patient died due to toxoplasmosis. Eight of these patients died during the first two decades. The third risk factor was year of transplantation. Although unrelated donor transplants have been used in a significantly increased number during the last decade studied and significantly older patients have undergone HSCT, the majority of patients in our study died during the first and second decades. The main causative pathogens were viruses (CMV n=18 and RSV n=1), fungi (n=12), or both viruses and fungi (CMV in combination with Aspergillus species or Candida species, n=4). These pathogens were found in autopsy and/or bronchoscopy (n=27), bronchoscopy alone (n=5), or sputum (n=3). The relative incidences during the three decades studied for CMV were 4.4%, 3.1%, and 1.1% and for fungi 3.0%, 2.0%, and 0.4%. We con-
cluded that even if death due to pulmonary complications still is a major cause of death after HSCT, the mortality rate due to early pneumonia has decreased over time. We regarded this finding as mainly a result of better diagnostic techniques, preventive strategies, and treatments. We also found that none of the patients who had received RIC (n=108) had died due to early pneumonia and that except for autopsy, bronchoscopy performed pre-mortem had been of diagnostic value since it had contributed to the diagnosis in 15 out of 27 (56%) cases.

<table>
<thead>
<tr>
<th>Cause of death within 3 months after HSCT</th>
<th>N=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>56</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>25</td>
</tr>
<tr>
<td>Fungal infection</td>
<td>16</td>
</tr>
<tr>
<td>Relapse</td>
<td>14</td>
</tr>
<tr>
<td>Septicemia</td>
<td>11</td>
</tr>
<tr>
<td>Multi organ failure</td>
<td>7</td>
</tr>
<tr>
<td>VOD/hepatic failure</td>
<td>6</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>2</td>
</tr>
<tr>
<td>PTLD</td>
<td>2</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>2</td>
</tr>
<tr>
<td>CLS</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory insufficiency</td>
<td>1</td>
</tr>
</tbody>
</table>

PTLD: Post transplant lymphoproliferative disease; VOD: Veno-occlusive disease; CLS: Capillary leak syndrome.

**Table 2.** Causes of death in 144 allogeneic HSCT patients, 1975-2003.

**Figure 7.** The cumulative incidence of early pneumonia-death A) in three 10-year periods; B) in patients receiving TcD graft versus a non-TcD graft; C) in patients with and without bacteremia.
5.2 THE DIAGNOSTIC VALUE OF BRONCHOALVEOLAR LAVAGE (STUDY II)

The second study was performed to verify if BAL, in case of pneumonia, really had been useful as a diagnostic tool for HSCT patients, as indicated in the first study. We also wanted to evaluate how and when this procedure should be performed to contribute to the diagnosis and if the procedure had contributed to a better outcome. In addition, we wanted to evaluate the value of cultures gathered from other fluids, such as sputum, nasopharynx, and blood. During the six years of the study (1998-2004), pneumonia was diagnosed 178 times in 167 out of 450 studied patients. Therefore, the incidence of pneumonia was 40%. However, BAL was only performed on 68 occasions (38%) in 57 of these patients. There were many reasons BAL was not performed in 110 patients (36 children) with pneumonia. For example, the patients’s condition was too poor, the symptoms were too discrete, other cultures had contributed to the diagnosis, or early administration of empirical antibiotics and recovery made the procedure unnecessary. However, in 33 cases the reason was unclear from a retrospective view. One of the reasons may have been that bronchoscopy had been regarded too difficult to perform on children because of their small airways and the frequent need of general anesthesia in children. In nine of the unclear cases (27%), the patients were children, which may have been one of the reasons for not performing BAL. However, previous reports support the idea that the benefits of BAL on children outweigh the risks and that BAL can be performed safely even in pediatric HSCT patients (298). In this study, only seven BALs were performed in children.

Of the 110 patients in whom a bronchoscopy was not performed, 32 died and eleven of these died due to pneumonia. An autopsy was performed on 21 (66%) of these patients. Based on autopsy results, a causative pathogen to the pneumonia was found in eight cases (Candida species, 3 cases; Aspergillus species, 1 case; Gram-negative bacteria, 3 cases; and CMV, 1 case), and based on blood cultures and sputum cultures two more causative agents were found (Streptococcus maltophilia and Pneumocystis jiroveci). In 78 patients without a bronchoscopy performed and who recovered from pneumonia, several pathogens were found in different cultures, mostly blood cultures (n=29). However, without positive cultures from the lungs, these findings were questioned as causes of the pneumonia.

BAL was considered to have contributed to the diagnosis in 43 (63%) cases. In 24 of these BALs, more than one pathogen was found. Since pneumonias may be caused by multiple pathogens, it was sometimes difficult to decide which one of the pathogens was most likely to be the causative agent. CMV, for example, can be accompanied with bacteria or Aspergillus species by superinfection and infection with Aspergillus species can be accompanied with bacterial agents, CMV, or zygomycetes co-infections. According to other authors, assessment should be thorough and cultures or other investigations indicating more than one causing pathogen should not be ignored (4). Of the 77 pathogens found in BAL, 53 were considered relevant (Table 3). In our study, several findings of Candida, enterococci, HHV-6, and other species were considered to be the result of colonization rather than causing pathogens, especially if other more relevant agents were found. According to some reports, however, these micro-organisms may have had a more important or amplifying role than first considered in the development of pneumonia (163, 216, 299). Sputum culture yielded the same result as the culture from BAL in 5/43 (12%) cases, nasopharyngeal aspirates in 3/43 (7%) cases, and blood cultures in 2/43 (5%) cases. This increases the doubts about the diagnostic value of cultures from specimens other than BAL. How-
ever, other cultures may be of value if BAL is not possible to perform. In 33 cases of pneumonia with negative cultures from other specimens, BAL yielded a trustworthy diagnosis. In BAL specimens, CMV was the most commonly pathogen found, mostly late (≥ 3 months) after HSCT. Furthermore, CMV was found in autopsy specimens from a patient who had had a negative BAL. In total, 9/12 patients (or 10/13 if the CMV patient without a BAL performed was included) died, the majority despite adequate antiviral therapy (n=7). On the other hand, 4/6 patients with aspergillus species in BAL at our center survived, possibly due to adequate antifungal therapy and an early diagnosis. Two more patients with *Aspergillus* pneumonia were found: one patient in whom a BAL was performed at another center (this patient died) and one in autopsy specimens.

The time and performance of BAL seemed to be important since the interval between radiographic infiltrations and positive BAL was shorter than in patients with negative BAL-findings (two versus six days), and since in four negative BALs the bronchoscope did not seem to have been positioned in the most affected segment as shown on chest X-rays. Furthermore, 92% of the patients with negative BAL and 79% of those with positive findings had broad spectrum antibiotics at the time of BAL, which may have influenced the outcome.

<table>
<thead>
<tr>
<th>Viruses, n=28</th>
<th>CMV 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV-6 1</td>
<td></td>
</tr>
<tr>
<td>HSV 2</td>
<td></td>
</tr>
<tr>
<td><em>Parainfluenza virus</em> 1</td>
<td></td>
</tr>
<tr>
<td>RSV 3</td>
<td></td>
</tr>
<tr>
<td><em>Influenza A virus</em> 1</td>
<td></td>
</tr>
<tr>
<td><em>Adenovirus</em> 3</td>
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<tr>
<td>EBV 2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungi, n=12</th>
<th><em>Aspergillus fumigatus</em> 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aspergillus niger</em> 1</td>
</tr>
<tr>
<td></td>
<td><em>Candida krusei</em> 1</td>
</tr>
<tr>
<td></td>
<td><em>Zygomycetes</em> 1</td>
</tr>
<tr>
<td></td>
<td><em>Pneumocystis jiroveci</em> 4</td>
</tr>
</tbody>
</table>

| Bacteria, n=13 | *Alpha streptococcus* 1 |
|               | *Streptococcus pneumoniae* 2 |
|               | *Hemolytic group B streptococcus* 1 |
|               | *Staphylococcus aureus* 1   |
|               | *Haemophilus influenzae* 1  |
|               | *Pseudomonas aeruginosa* 2  |
|               | *Escherichia coli* 1        |
|               | *Enterococcus faecalis* 1   |
|               | *Enterococcus faecium* 1    |
|               | *Chlamydia pneumoniae* 1    |
|               | *Nocardia* 1                |

Table 3. Micro-organisms found in BAL (n=53) and considered as causative in 43 cases.
Despite the high diagnostic yield of BAL and despite the fact that this procedure contributed to changing the therapy in 32/43 patients, the mortality was higher with a BAL performed (38%) compared to without this procedure (29%). The explanation may be that there were fewer children, who are more prone to recover, in the BAL group and that the patients with a BAL performed, most often had pathogens that were more difficult to treat.

5.3 DIAGNOSTIC PROCEDURES AND RISK FACTORS FOR BO (STUDY III)

Pulmonary function tests are highly dependent on patient cooperation and therefore this procedure can be difficult to perform in patients who are in a bad shape or unable to comprehend and/or follow the instructions. For example, for children < 6 years of age, PFTs are most often difficult to evaluate. Another limitation, if the spirometry is only executed very occasionally, is that the raw values may be normal despite a beginning respiratory complication and may therefore not be detected at an early stage. PFTs are more useful as a monitoring tool: a sudden decrease in FEV\(_1\) or other spirometric measures in the same patient can signal worsening control even if the raw value is still normal. In the third study, 1177 spirometries performed in 330 patients were re-evaluated by both an experienced physiologist (Thomas Gustafsson) and a lung specialist (Ulrica Forslöw). Further 197 patients, who had undergone HSCT during the same period (1995-2003), were included in the study material but in those patients spirometry data were not available. The causes of no available PFTs were that 77 patients died within 120 days after HSCT and thus were in a too poor of a condition for further diagnostic investigations such as PFTs, 102 patients had insufficient follow-up data due to either living in another country and/or lack of PFTs, and 18 patients were < 3 years of age. We aimed to find out the incidence, risk factors and outcome of BO that is characterized by a progressive and persistent AFO. Therefore patients with mild, moderate or severe AFO (FEV\(_1\) 66-80%, 51-65%, or \(\leq 50\%\)) were sorted out for a more thorough investigation of clinical, radiological, pathological and laboratory data. In previous studies, the reported incidence has varied a lot, mainly depending on the former lack of uniform criteria (155). Therefore, we tried to follow thoroughly the criteria for BO according to the NIH consensus document (245). The cumulative incidence of BO at our center was 4.8% (Figure 8).

![Figure 8. Cumulative incidence of BO at Karolinska University Hospital, Huddinge 1995-2003 following HSCT.](image-url)
Four of the 25 patients who were considered BO cases were children 6-13 years old. In most of the patients, PFTs were performed repeatedly each three to six months after HSCT. In total, 176 PFTs were performed (median of 7 (range=2-14) times) in these 25 patients. At the time of BO, diagnosis of the median values of FEV\textsubscript{1}, FEV\textsubscript{1}/FVC, FEF\textsubscript{50}, FEF\textsubscript{75}, RV, TLC, and DLCO were 49\%, 61\%, 25\%, 18\%, 177\%, 97\%, and 59\%, respectively. In seven patients, the FEF\textsubscript{75} value was reduced already in the spirometry preceding the spirometry that established the diagnosis (according to the NIH criteria). Dynamic spirometries are easier and more possible to perform than static spirometries and DLCO tests. FEV\textsubscript{1} was considered to be the most reliable and easy parameter to detect BO and to monitor the development of BO. FEF\textsubscript{50} and FEF\textsubscript{75} were also considered to be of interest. Even if the raw values of FEF\textsubscript{50} and FEF\textsubscript{75} are rather non-specific and depend on the lung volume, intra-individual comparisons can serve as an early warning of BO. In these patients, radiographic data were also studied. Several high resolution CTs (HRCTs) (n=50) were performed in the majority of BO patients. However, the diagnostic value of these was limited since only eight (32\%) patients had changes associated with BO as bronchiectasies with thickened and dilated bronchi, and/or air trapping and a mosaic pattern. The median time from HSCT to development of BO was 356 (84-1823) days. Patients who developed BO within or after one year were defined as having an early or late BO, respectively. When comparing the course and outcome of disease in patients with early versus late BO, we found that the mortality was higher in those with early (60\%) than those with late BO (20\%). Thus, the five-year survival for patients with late-onset BO was high in our study. This might have depended on a better immune competence late rather than early after HSCT and/or that patients with late BO had had a protracted immunosuppressive treatment due to GVHD compared to those with early BO (Figure 9).

**Figure 9.** Cumulative proportion of survivors in patients with early (< 1 year onset of BO after HSCT) compared to late (> 1 year) BO.
The majority of patients with early BO had a rapid decline in lung function, whereas others, mostly patients with late onset BO, had a more protracted course. However, regardless of whether the onset was early or late, the survivors ended up with equally poor lung function. Risk factors given in the multivariate regression analysis were cGVHD (p<0.001) and no DLI treatment (p=0.03) (Figure 10). These factors were found to be significantly associated with development of BO even when treated as time-dependent variables before introduction to Cox proportional hazards model. While cGVHD is a well-known risk factor for BO as shown in several previous studies (255, 256, 262, 300), DLI having a potential preventive role was an unexpected finding. DLI was administered to 126 patients (24%), and two of them developed BO. DLI changes the cytokine profile towards a Th1 profile and has been shown to clear pre-existing infections or to protect from development of new infections. BO, on the other hand, is strongly associated with cGVHD, a condition believed to cause tissue damage through a Th2 profile. Could the change towards a Th1 profile also protect the patient from non-infectious diseases? The majority of patients who received DLI in our study (53%) survived for more than one year after DLI.

Figure 10. Cumulative incidence of cGVHD and DLI, risk factors for BO.

5.4 THE CONDITIONING’S IMPACT OF DEATH FROM PNEUMONIA (STUDY IV)

In our first study we found that none of the patients who had received RIC died due to early pneumonia. Therefore, the question was raised whether the choice of conditioning regimen influenced the decreased fatal outcome in these patients. During the nine years of study (2000-2009), 68 patients (17 children ≤ 16 years) were identified as having died due to pneumonia, 22 of these died within 100 days (early) after HSCT. Thus, the cumulative incidence of death due to pneumonia was 10.5% and due to early pneumonia, 3.2%. Two hundred and five patients (30%) died due to other causes than pneumonia, and 45 of these patients (6.5%) died within 100 days after HSCT. However, in patients receiving MAC the cumulative incidence of death from early pneumonia was 4.2%, which was significantly higher than in for those receiving RIC (2.1%) as a conditioning regimen (Figure 11).
A total of 59 supposed causative pathogens were found in 47 (69%) of the patients who died due to pneumonia. In 22/34 patients who received MAC and in 25/34 who received RIC, 28 and 31 pathogens were found, respectively. In nine MAC and twelve RIC patients, the same pathogens were found in cultures from different specimens. The diagnostic yield of especially autopsy and bronchoscopy was high, since autopsy, which was performed in 36 patients, contributed to the diagnosis in 26 of these (72%), and bronchoscopy, which was performed in 17 patients, contributed to the diagnosis in eleven out of 17 (65%). In MAC and RIC patients, viruses, fungi, and bacteria were alone or in combination causative pathogens: viruses in each eight cases (of which eleven of these were CMV), fungi in 16 and 13 (of which 16 were Aspergillus spp) cases, and bacteria in four and ten cases. Fifteen of the MAC-patients and seven of the RIC patients died due to early pneumonia. In those patients, eleven and seven pathogens were found, respectively. For MAC and RIC, viruses were found in four and one case (of which three were CMV), fungi in six and two cases (of which three were Aspergillus spp) and bacteria in one and four cases. Of the 59 causative pathogens found, 16 (27%), 29 (49%), and 14 (24%) pathogens were of viral, fungal and bacterial origin. Only two of the patients who died early due to bacteria, one with MAC and one with RIC, had neutropenia. Risk factors given in the multivariate analysis for early death from pneumonia were a second HSCT, bacteremia, and MAC. A second allogeneic HSCT has been given to patients with graft failure or relapse. However, this procedure also makes the patients more susceptible to infections by toxic organ side effects and severe damage to the bone marrow stroma and immune system than if HSCT is only performed once. Another finding was that administration of MSCs was associated with an increased risk of overall pneumonia death (but not early pneumonia death). MSCs were given to 53 patients as treatment for GVHD (n=22), HC (n=12), as engraftment support (n=16), and other reasons (n=3). In a study from our group, a high risk of death due to infections was found in patients

Figure 11. Cumulative incidence of early death from pneumonia in patients receiving MAC and patients receiving RIC.
receiving MSCs due to severe GVHD (von Bahr et al. manuscript). In another report, indica-
tions of an inferior thymus T-cell output were found in patients receiving MSCs as engraftment
support for CB transplantation (Uhlin et al. manuscript). Altogether, these findings indicate a
future need for an even more cautious attitude toward MSCs than we already have.
6 CONCLUSIONS

1. Pneumonia, especially of viral and fungal origin, remains one of the most common causes of morbidity and mortality after HSCT, although the rate of mortality has decreased at our center. This is probably a result of improved diagnostic, prophylactic, and therapeutic methods and treatments.

2. Bronchoalveolar lavage is a diagnostic method with high diagnostic yield and few complications. The diagnostic yield was even higher if the procedure was performed soon after detection of radiographic infiltrations, compatible with pneumonia, and with the bronchoscope positioned correctly in the most affected segment according to the radiograph signs.

3. Most cases of pneumonia due to CMV and Aspergillus species were established with BAL before death and would not have been found premortem without this diagnostic procedure.

4. The efforts to identify pathogens causing pneumonia may result in a changed and more directed treatment, thereby avoiding unnecessary, expensive, and potentially toxic drugs.

5. In the first study, risk factors for death from early pneumonia were: year of transplantation, bacteremia and receiving TcD graft. In the fourth study, risk factors (in more recent years) were: receiving a second HSCT, bacteremia, and MAC. TcD graft is no longer used as an immunosuppressive method. A second HSCT and a non-myeloablative conditioning regimen are strategies that have been increasingly used in recent years. Bacteremia, however, remain one of the most important risk factors for death from early pneumonia for the entire study period. In patients receiving RIC, in itself a strategy that reduced the risk of early pneumonia, the finding of bacteria as the cause of death was somewhat higher than for patients receiving MAC. Therefore, even more efforts should be made to improve diagnosis and to prevent bacteremia.

6. MSCs, another new treatment strategy used especially for patients with severe acute GVHD or HC but also as engraftment support, should be used with caution since this treatment was also indicated as a potential risk factor for overall pneumonia death.

7. The RIC regimen seems to reduce death from early pneumonia, but not death from overall pneumonia. More attention should be directed towards the risk of death due to late pneumonia, hopefully leading to a more thorough follow-up, also in later stages, when signs of pneumonia occur.

8. Other pulmonary complications, such as BO, are also important contributors to morbidity and mortality after HSCT. Regular pulmonary function tests should be performed, especially dynamic spirometries, to detect BO as early as possible and then to monitor its development. Even if the results of treatment are rather unfruitful, it may be of some benefit, including temporarily stabilizing the disease, a finding from the third study.

9. Two categories of BO were seen: Patients with a late (> 1 year) onset of BO had a slower decrease of FEV₁ and a better prognosis than patients with development of BO within one year. In patients with a late onset of BO, the five-year survival was 80% compared
to 40% in those with an early onset. However, regardless of early or late development of BO, the survivors ended up with equally poor lung function.

10. One of the risk factors seen in the multivariate analysis was, as expected, cGVHD. However, the other risk factor was unexpected, namely not receiving DLI, which means that DLI may have had a protective role against development of BO.
7 FUTURE PERSPECTIVES

All the studies were retrospective and included a very large number of allogeneic HSCT patients. There is always a risk when performing such studies since some data may be lacking and since the patient files do not always contain the data or results. Nevertheless, these kinds of studies are of utmost importance for evaluating what was done right or wrong and what would have been possible to perform better. Diagnostic strategies and treatments and risk factor analyses demand extensive patient materials, such as those we have had the opportunity to handle. A thorough evaluation of the diagnostic yield of procedures, such as BAL or results of spirometry testing, and of the most optimal way of performing these kinds of investigations, should be the basis for written documents and check-lists used whenever a pulmonary complication is suspected. Such an approach should detect issues and suggest a treatment strategy at an early stage.

Even if the diagnostic and treatment strategies concerning infectious complications, including bacterial but also fungal and viral pneumonia have improved, the outcome for allogeneic HSCT patients over time is less than optimal. These studies asked, among other questions, whether pathogens normally regarded more as colonizers than as causative agents play an important role for the course and outcome of infectious complications. For example, enterococci species were abundant, especially in autopsy specimens. Perhaps future studies should investigate this phenomenon more carefully. Mucositis and the use of opiates and sedatives may predispose to aspiration of secretions that contain various bacterial and fungal pathogens. One of the theories of what may cause non-infectious complications, such as BO, is GVHD associated esophagitis. Furthermore, bacteremia was shown to often be the start of pneumonia. What happens in the lungs’ micro-environment in these most often severely immunocompromized patients when toxic damage is present due to tough conditioning regimens, especially in case of aspiration of otherwise safe colonizers? Does the non-infectious complication perhaps start with an infection?

The alveolar macrophages (AMs) are phagocytes that play a critical role in homeostasis, host defense, the response of foreign substances, and tissue remodeling (301). They are highly adaptive in the innate immune system and can be specifically modified to whatever functions needed depending on their state of differentiation and micro-environmental factors encountered. AMs release numerous secretory products and interact with other cells and molecules through the expression of several surface receptors. When insufficient to ward off the threat, alveolar macrophages can release proinflammatory cytokines and chemokines to call for cells responsible for the adaptive immune response (302). The adaptive immunity is suppressed by AMs’ effects on interstitial dendritic cells, B-cells and T-cells, as these cells are less selective of what they destroy, and often cause unnecessary damage to normal cells. To prevent uncontrolled inflammation in the lower respiratory tract, AMs secrete nitric oxide, prostaglandins, IL-4, and IL-10 and transforming growth factor beta (TGFβ) (303-305). However, in patients with immunoregulatory disturbances in the lungs after HSCT, these mechanisms should be impaired, especially within 100 days after HSCT, which perhaps pave the way for pathogens to set normal lung defense mechanisms out of play (306). At later stages, the defense mechanisms begin to restore and perhaps the patient achieves more of a Th2 response to colonizers, perhaps leading to more of a chronic destruction of the lung tissue.
Furthermore, previous studies have shown that TNFα mediates GVHD by amplifying donor immune responses to host tissues and by direct toxicity to target organs (307, 308) and that TNFα also is an important effector molecule in the development of IPS (234, 241, 309). Other studies comparing infectious (pneumonia) with non-infectious pulmonary complications (IPS, BO) have shown that TNFα is significantly expressed in both groups (250). Recent studies have shown promising results of treatment with the TNFα inhibitor Etanercept for GVHD (240, 310, 311). Regarding BO an early detection of patients at risk is probably important for the outcome. Low or decreasing levels of Clara cell 16 kDa secretory protein (CC16) has been found to be associated with BO (Mattsson et al, 2005).

Further prospective BAL or lung tissue studies could perhaps contribute to a better understanding of the actions and interactions of cells active in immune defense and the course of events in the lungs that leads to disturbed lung defense and finally to death due to both infectious and non-infectious pulmonary complications. Perhaps prospective studies could be designed to find a way to restore the impaired function of AMs and to find out whether the colonizers should be diagnosed and counteracted earlier and more actively than today. A better understanding of these mechanisms and the elaboration of effective investigation and monitoring tools could hopefully lead to new treatment strategies and to an improved ability to predict the outcome for individual recipients and thereby to tailor treatment strategies for these individuals in the future.
8 ACKNOWLEDGEMENTS

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REFERENCES


312. Renjith Krishnan is the creator of the digital photo covering the front.