Prediction and prevention of infectious complications in children with cancer
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Chapter

Introduction

Infectious complications in children with cancer: Prediction, prevention and management
Introduction

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1 General introduction

1.1 Epidemiology of infections in oncology patients:

Improvement has been made over the past 30 years in cure rates for pediatric oncology patients. This is now estimated around 70%.

With the intensification of chemotherapy, the need for adequate supportive care is of utmost importance to maintain this high percentage of survival. As therapy becomes more intense infectious complications play a major role. In the 60's Bodey et al showed that the risk for infections increased rapidly if the granulocyte-count dropped below 500 cells/mm\(^3\). This study showed an incidence of severe infections of 43 episodes per 100 admitted patients if the granulocyte count was below 500 cells/mm\(^3\), compared to <5 infectious episodes if the neutrophil count was >1500 cells/mm\(^3\). The fatality-rate of bacterial infections in those early years was extremely high, but with the introduction of empiric antibiotics the infection mortality rate has dropped to 4-6% of all new adult oncology patients, and 0.6-1% of all new pediatric oncology patients. The neutropenic patient with fever forms a heterogenous group regarding infection and risk of complications, depending on the chemotherapy given and the underlying malignancy. Of these patients 12-17% will develop a definite proven bacteremia or fungemia.

The pattern of infectious micro-organisms has changed significantly over time. Gram-positive organisms prevalent in the 1950's and the 1960's, showed a drop in incidence 10 years ago with Gram-negative organisms increasing. Towards the end of the 90's the Gram-positive organisms are most prevalent again. Gram-positive organisms are isolated in 15% of febrile episodes and cause 60-70% of proven bacteremia's, while fungal infections are documented in 2-8% of all bloodstream infections. Of the Gram-positive organisms the coagulase-negative staphylococci (CNS) are the most common (30%), i.e half of the proven bacteremia's. Enterococcal (7%) and viridans-group streptococcal species (10%) are becoming problematic, because of the increasing antibiotic resistance. Of the Gram-negative organisms the most frequent are Escherichia coli (8%), Klebsiella spp (6%), Serratia spp (3%), Proteus spp (3%). and Pseudomonas aeruginosa (5%).

In the 1990's, patients receiving chemotherapy showed an increased risk of opportunistic infections, probably secondary to the use of dose-intensified chemotherapy. In the past, the greatest toxic risks were related to neutropenia and hemorrhage. The intervals between chemotherapy courses were determined by the time necessary to achieve safe recovery of neutrophils and platelets. With the use of hematopoietic cytokines, chemotherapy is no longer limited by neutropenia or thrombocytopenia and many patients are receiving significantly higher dosages of chemotherapy more frequently as compared to historical controls. This decreased interval between chemotherapy courses may provide inadequate time for lymphocyte recovery and could contribute to an increased risk of prolonged immunodeficiency. With the more intensive chemotherapeutic protocols and bone-marrow transplantation other serious infections
emerge because of the prolonged severe neutropenia. In these patients fungal organisms occur such as *Candida* spp, *Aspergillus* spp or other opportunistic fungi. They also have a poor cellular immune function and are susceptible to infections caused by intracellular pathogens, mycobacteria and *Listeria monocytogenes*, *Cryptococcus neoformans*, viruses such as cytomegalovirus, adenovirus, herpesvirus and varicella-zoster virus, and protozoa among which *Pneumocystis carinii* is the most common.

1.2 Management of infections in neutropenic patients

In the management of all febrile neutropenic patients the clinician is dedicated to careful and repeated evaluation for specific signs and symptoms of a focus or type of infection. This is of prime importance in caring for the febrile neutropenic patient. Many guidelines have been developed to offer these patients maximal care. Because the progression of infection in neutropenic patients can be rapid, empirical therapy should be administered promptly to all neutropenic patients at the onset of fever, where fever is defined as a single oral temperature of $\geq 38.3^{\circ}\text{C}$, and neutropenia is defined as $< 500$ cells/ mm$^3$. The latest update of these guidelines has been in 2002 by Hughes et al. All recommendations are made on base of scientific data and peer reviewed information, but it must be realized that in treating the individual patient, guidelines are not sufficient and optimal patient care will include repeated clinical examination, thoughtful consideration of the microbiological data, and recognition of institutional trends, and adaptation of the guidelines if needed. Three general schemes are considered a) monotherapy b) duotherapy without vancomycin, c) vancomycin plus one or two drugs. Antibiotic treatment for at least 3-5 days is usually required to determine the efficacy of the initial regimen. Even when the patient remains febrile the clinician may wait 5 days to make any changes in the antimicrobial regimen, unless there is clinical deterioration or a positive blood-culture result. If fever persists after 5 days and there is profound neutropenia, 1 of 3 choices of management should be made. 1) continue treatment with the initial regimen, this can be considered if the patient remains otherwise stable, 2) change or add antibiotic treatment, this can be considered if during the first days it becomes clear that there is a focus of infection, such as neutropenic enterocolitis and Clostridium toxin is found positive then oral Flagyl should be added. 3) the third choice to consider is the addition of anti-fungal therapy. Amphotericin B is the first drug of choice. Every effort should be made to determine whether systemic fungal infection exists. The 4th option to stop all intravenous antibiotics should not be considered. Concerning the duration of antibiotic therapy the evidence is not very strong. Most approaches recommend stopping antibiotics when the patient has been afebrile for 48 hours, and there should be evidence of marrow recovery. If anti-fungal treatment has been started it is recommended to continue this for 14 days if no positive fungal culture was found, and in case of a positive fungal culture at least 21 days is recommended or until all signs of fungal infection are controlled.

In the last decade there is a better understanding of the syndrome of febrile neutropenia,
including the development of risk prediction rules, and risk-based strategies (see paragraph 2). This is used in adult oncology patients, not yet in pediatric oncology patients. Even though the spectrum of febrile neutropenia is more clear severe infections do occur. Two of these serious infectious complications will be discussed.

1.2 Specific infectious complications

1.2.1 Neutropenic enterocolitis

Neutropenic enterocolitis is defined as a necrotizing inflammation of the colon in a severely immunocompromised patient \(^{18}\). This is considered one of the life-threatening complications related to bone marrow suppression and neutropenia. The incidence ranges from 5-40% in severe (absolute neutrophil count < 100 cells/mm\(^3\)) neutropenic patients\(^{14,19}\). The mortality with good medical management has been estimated at 20% \(^{19}\). Although any part of the gastro-intestinal tract may be involved, the cecum appears to be the most severely affected with mucosal ulceration, gangrene, and perforation. Some investigators suggested that the cecum is more prone to this injury due to its greater distensibility, relative lower blood flow, and increased stasis of luminal contents compared to the rest of the gastrointestinal tract \(^{20}\).

The presence of micro-organisms such as *Clostridium difficile*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., and *Candida* spp in areas of necrotic bowel and in blood cultures from affected patients suggests that enterocolitis is primarily an infectious process. The primary mucosal insult that allows bacterial invasion may result from a number of mechanisms, including chemotherapy-induced mucosal injury, shock leading to low flow and mucosal ischemia, abnormal intestinal flora secondary to aggressive, broad-spectrum antibiotics, or necrosis of tumor infiltrates. The invasion of bacteria itself can cause further necrosis of the bowel wall, leading to full-thickness infarction and perforation of the intestine.

Affected patients typically present with fever, abdominal pain and distension, and diarrhea. It must be realized that the clinical presentation of neutropenic enterocolitis can be extremely variable, and that there are no strict criteria to make the diagnosis. Furthermore, the symptoms are nonspecific and may be similar to those of a number of gastrointestinal processes. There are no specific laboratory or radiologic findings that form the golden standard for diagnosing neutropenic enterocolitis. Plain abdominal radiographs and ultra-sound may demonstrate dilated loops of bowel, thickening of the bowel wall, “thumb printing” resulting from bowel wall edema, or indications of a right lower quadrant mass or phlegmon. Pneumatosis intestinalis can be seen and is not an indication for surgical intervention \(^{16,21}\). Free intraperitoneal air indicates perforation of the bowel wall, this is an indication for surgical intervention. The initial treatment for neutropenic colitis is supportive, with the administration of broad-spectrum antibiotics, intravenous fluids and bowel rest. It is extremely difficult to predict the course of the disease. Therefore in Chapter 4 of this thesis a prospective study is presented to gain insight in this severe complication and to identify markers for severity.
1.2.2 Varicella zoster infection

Varicella (chickenpox) is an acute highly infectious disease caused by VZV. Children generally develop mild disease, manifested by fever, a vesicular rash and mild constitutional symptoms. However, in children with malignant disease the incidence of complications and even mortality due to varicella infection is high. The complication-rate is approximately 30% and in untreated cases the mortality-rate approaches 20% 22. Antiviral therapy has improved the outcome considerably but the overall mortality rate in the immuno-compromised patient remains 7% 22,23. The most common complication is acute secondary bacterial skin infection, caused by Staphylococcus aureus or Streptococcus pyogenes. In children under 5 years of age, there is an increase of this complication even leading to a streptococcal toxic shock syndrome 24. Neurologic complications can also occur in 1-3 patients per 10,000 cases 25. The most common is postinfectious cerebellar ataxia which occurs in about 1 in 4000 varicella cases 25 which most often resolves without complications. Meningoencephalitis occurs slightly less frequent (1-2 episodes per 10,000 varicella cases) but has a less favorable outcome 26. The mortality rate ranges from 5-25% and neurologic sequelae are seen in 20% of patients 27. Other complications are pneumonia, visceral disorders (including hepatitis and severe gastro-intestinal symptoms), hematological problems (thrombocytopenia, pancytopenia) and the development of hemorrhagic varicella.

As awareness of the morbidity and mortality due to varicella infection became established, the interest in the live-attenuated vaccine increased 28. The varicella vaccine was found to be safe, immunogenic and effective in leukemic children 29,30. This VZV vaccine was given in the maintenance phase of chemotherapy and chemotherapy was delayed at the time the vaccination was given. Therefore in chapter 7 an ongoing prospective study will be presented administering varicella vaccine to pediatric oncology patients (both patients with hematological malignancies as patients with solid tumors) in an early phase of their disease, without delaying the chemotherapy, to evaluate the efficacy of VZV vaccine in an early stage of chemotherapy treatment.

2 Predictors of the clinical course of infection

2:1 Risk-assessment and infection

Different approaches have been developed over time regarding the empirical antimicrobial therapy for fever in neutropenic patients. It is now known that the febrile neutropenic patient forms a heterogenous population, constituting a group at low-risk of serious complications and a group at high-risk of serious complications. Many studies have been done focusing on risk-assessment in the febrile neutropenic patient. If a low risk group is identified this opens the possibilities to different treatment strategies including outpatient antibiotic therapy after early discharge from the hospital, or outpatient therapy for the entire febrile episode, using
parenteral, sequential (intravenous followed by oral) or oral antibiotic regimens. The trials on adult patients \(^{31,32}\) have validated a clinical scoring system to identify the different subgroups of patients. Talcott validated a clinical model for predicting the medical risk for infection in adult oncology patients with fever and neutropenia \(^{31}\). Stepwise logistic regression analysis of presenting clinical characteristics was performed to model the independent predictors of subsequent medical complications. Inappropriate candidates for early discharge are patients with fever and neutropenia who are already ill and hospitalized (Group I), newly ill patients (out-patients with serious concurrent co-morbidity, Group II), or at high risk of progressive cancer (out-patients with uncontrolled cancer, Group III). Clinically stable patients without co-morbidity and without serious complications (Group IV) constitute the group that can be considered at low risk for serious medical complications. Around 40% of all febrile neutropenic patients belong to Group IV. Klastersky et al \(^{32}\) refined the Talcott model and validated an international clinical prediction rule, based on patient history, age, outpatient status, acute clinical status and severity of disease. The clinical prediction rule derived from the score identifies a low-risk group of patients with a score of at least 21. This threshold corresponds to a positive predictive value of 94%. This

<table>
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<th>burden of illness</th>
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<td>symptoms non-existent or mild</td>
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</tr>
<tr>
<td>symptoms moderate</td>
<td>3</td>
</tr>
<tr>
<td>absence of hypotension</td>
<td>5</td>
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<tr>
<td>(systolic bloodpressure&lt;90 mmHg)</td>
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</tr>
<tr>
<td>absence of chronic pulmonary obstructive disease</td>
<td>4</td>
</tr>
<tr>
<td>solid tumor or no previous fungal infection</td>
<td>4</td>
</tr>
<tr>
<td>absence of dehydration</td>
<td>3</td>
</tr>
<tr>
<td>outpatient status</td>
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<tr>
<td>age&lt;60 years</td>
<td>2</td>
</tr>
</tbody>
</table>

threshold was a compromise between a safe positive predictive value, and a misclassification rate that would not be too high. This model can make up more than 60% of all febrile neutropenic patients, it is however not known yet if this model will retain its high predictive values in a setting of out-patient management with intravenous or oral antibiotics. The management of febrile neutropenia in children with cancer has not yet led to an international adapted standard clinical prediction rule identifying the children at low risk of serious infections and those at high risk for serious infections. Many pediatric trials on this subject have been performed. Orudjev et al \(^{33}\) reported on all pediatric trials done on risk-assessment. Twenty-seven prospective trials were identified and five reviews. Orudjev divided studies that concentrated on clinical comorbidities ruling out children suitable for the low-risk strategy and studies concentrating on laboratory parameters.
The patient related co-morbidities ruling out children as being classified "low-risk" are:
age<1 year (limited data), age <5 years (limited data),
history: bacteremia during previous neutropenic episodes, rigors after flushing the central venous catheter, non-compliance, >1-2 hours from hospital
medical conditions requiring hospitalisation: shock, metabolic instability, altered mental status, hemorrhage, dehydration, pneumonitis, mucositis, increased work of breathing, perirectal or soft tissue abscesses, diarrhea, vomiting, irritability, organ failure
cancer-associated co-morbidities: uncontrolled tumor, leukemia at diagnosis, leukemia in relapse
treatment-associated comorbidities: anticipated neutropenia >7 days, 1-12 months post stem-cell transplant (limited data)

Information on demographic findings such as age and underlying cancer is limited. Infants were excluded from a number of studies, and 2 randomized trials on oral antibiotics excluded children <5 years of age 34,35. If we consider most of the co-morbidities mentioned, it will be clear that on admission a thorough history and physical examination are needed to classify the patient in the "low-risk" or "high-risk" category.

Next to studies on clinical parameters, studies have focussed on laboratory parameters to classify the pediatric febrile neutropenic patient at "low-risk" or "high risk" of infectious complications. Of the laboratory parameters two were found significant, the absolute monocyte count on admission of the patient and the C-reactive protein (CRP). If the absolute monocyte count (AmoC) was above 100 cells/mm³ and the CRP was below 90 mg/L the risk for bacteremia was low (5%) 36-38. A consistent trend has been shown in patients with a low neutrophil count (<100 cells/mm³) or no evidence of marrow recovery constituting the "high risk" group. If by day 4 of febrile neutropenia, the plateletcount rises and the monocyte count is >100 cells/mm³, then this constitutes marrow recovery and patients can be defined as "low-risk" patients. The "low-risk prediction rule" in pediatric patients is not a clear-cut rule. Mullen et al 39 has found that of the 50% "low-risk" patients, one third were not eligible for out-patient therapy because of non-medical reasons, such as organizational and logistic variables in treating the patient as an "out-patient", insecure parents who prefer the child to be admitted for the duration of the fever.

In many instances, empirical therapy will be instituted at the hospital and decisions can be made to switch to oral antibiotic therapy after initial intravenous therapy. In pediatric oncology this is not the standard of care as yet, but the above trials offer support for reconsidering the broad-spectrum antibiotics started intravenously, which might lead to less antibiotic usage and less days in hospital.

Within the "high-risk" group of patients who need hospitalisation because of the existent co-morbidities or/and laboratory parameters, it is important to identify within this group of patients the patients at high risk for serious complications of infections such as neutropenic enterocolitis.
The predictive value of the measurement of inflammatory markers in serum and plasma has been evaluated. To understand this first a basic overview of the immunological system will be presented.

2.1 The immunological system, a basic overview

The reaction of the immune system towards certain triggers from the in- or outside environment consists of an innate immune response (non-specific) and an adaptive (specific) immune response. The innate immune response is considered the first line of defense, and sets the stage for the adaptive specific response. The protective effects are a result of the steady-state resistance caused by physical barriers like the skin and mucous membranes. Apart from acting as a barrier, the skin and mucous membranes also have effective antimicrobial properties. After the microorganisms invade the epithelial barrier the cells of the innate immune response play a crucial role in the initiation and subsequent direction of the adaptive immune response. There is a delay of 4-7 days before the adaptive immune response takes effect; therefore the innate immune response has a critical role in the control of infections during the first few days. All components of innate immunity are present prior to exposure to micro-organisms and will act immediately towards them.

The main component of soluble factors belonging to the innate immunity is the complement system. This is a group of 20 or more serum proteins that interact in an orderly fashion and are referred to as the complement cascade. The effector functions of complement can be activated by 3 pathways (figure 1). The classical pathway, activated by antibody binding to antigen. The lectin pathway initiated by binding of lectins (such as mannan-binding lectin (MBL)) to mannose-containing proteins or other carbohydrates on bacteria and viruses, and the alternative pathway when a spontaneously activated complement component binds to the surface of a pathogen. The early events of all three pathways lead to a number of cleavage reactions ending in the formation of so called C3 convertase. From there on, a cascade of enzymatic activities occur, leading to complement-binding to receptors on phagocytes followed by opsonization direct lysis of the micro-organisms, and the induction of peptide mediators of inflammation, such as C3a and C5a.

Mononuclear phagocytes in blood, lymph nodes, spleen, liver, bone-marrow, und lung constitute the reticulo-endothelial system. These cells may recognize microbes by pattern recognition receptors on the surface of the macrophage, or receptors for IgG on complement fragments deposited on the microbes upon opsonization. Engagement of many of the pattern recognition receptors lead to microbial clearance, and lead to the production of cytokines and chemokines by mononuclear phagocytes and dendritic cells. Mononuclear phagocytes are important producers of the pro-inflammatory cytokines such as, TNF-α, interleukin IL-1β, IL-12 and IL-18, which influence the inflammatory response and provide priming signals for induction of adaptive immunity. Macrophages produce modulatory factors, such as IL-10 and TGF-α, which inhibit the production and action of the pro-inflammatory cytokines, in particular when actively clearing
Pathways of Complement Activation

**Classical pathway**
- Binding to IgM and IgG and microbial surfaces
- C1q
- C1r
- C1s
- Activation of C4 & C2
- MBL
- MASP-1
- MASP-2
- MASP-1?
- C3 activation

**Lectin pathway**
- Binding to carbohydrate on microbial surfaces
- Factor B
- Factor D
- Properdin

**Alternative pathway**
- Binding to surfaces upon autoactivation
- C3b

Figure 1: Complement-cascade demonstrating the 3 pathways of activation, the classical pathway, the alternative pathway and the lectin pathway

Figure 2: The cell-mediated immune response and the role of the T-helper cell
apoptotic material \(^{41,42}\). Apart from the macrophages, tissue cells are also able to produce cytokines, such as IL-8 an important mediator of polymorphonuclear leucocytes (PMN) localization, together this will regulate the onset of the adaptive immunity\(^{43,45}\).

The adaptive immunity consists of specific humoral immunity and cellular immunity. The adaptive humoral response to infection involves the production of specific antibodies by B lymphocytes, the binding of these antibodies to the pathogen and elimination of the pathogen by cells of the humoral immune system. B-cell activation requires the binding of a specific antigen to the B-cell surface immunoglobulin (i.e. antigen receptor) and the interaction of the naïve B cell with antigen-specific T-helper cells. These interactions occur in the lymph nodes draining on the tissues. These T-helper cells induce B cell proliferation after which the naïve B-cells differentiate in antibody-secreting plasma cells or memory-B-cells, that may leave these lymph nodes and settle in other secondary lymph organs, such as the spleen, Peyer's patches or the bone-marrow. Antibodies enhance complement and granulocyte mediated killing of the invading free-living pathogens.

The cell-mediated immune response is responsible for destruction of organisms that can not be neutralized by antibodies (such as many viruses, protozoa, parasites and some intracellular bacterial pathogens).

When cells are recognized as "non-self" by T-cells, they are lysed by T-lymphocytes, exposing the pathogen to antibodies, complement and granulocytes, capable of eliminating the pathogens. T cells are functionally divided in CD4+ T lymphocytes also called T-helper cells and CD8+ T cells generally known as cytotoxic T cells. CD8+ T cells play an important and active role in the control of viral infections by lysing virus-infected cells but are also important in eliminating intracellular pathogens. CD4+ T cells not only function as helper cells for specific antibody generation, but also play a role in the recognition of antigen-presenting cells resulting in lymphokine release as the principal effector function (figure 2), as well as activating the macrophages to perform optimal killing. In this latter reaction, IFN-γ plays an essential role in case of mycobacterial infection and salmonellosis \(^{43}\).

2.2 The human genetics of infection, the role of MBL

The course of many infectious diseases is influenced substantially by genetic variation in the host. For instance the association of cholera symptoms and bloodgroup O has been found consistently in several studies \(^{46,47}\). It is known that innate immunity plays a critical role in the first few days of infection. Mutations and polymorphisms in genes encoding members of the innate immune system appear to alter the host susceptibility and responses to various pathogenic micro-organisms. The most well known is the sickle cell trait protecting against Malaria falciparum, other examples are tuberculosis susceptibility linked to variations in the \(NRAMP1\) gene, a HLA-DQ allele, and IL-12 deficiency \(^{48,49}\), susceptibility to pneumococcal disease linked to defective production of antibody to the pneumococcal capsule \(^{50}\). This list will extend in the future and offer possibilities for treatment and vaccination.
One of the essential components of the innate immune system is the complement system, which has 3 possible routes of activation, as described earlier. One of these routes is the MBL pathway. MBL is a circulating protein that recognizes a wide range of micro-organisms, including certain Gram-positive and Gram-negative bacteria, yeast, fungi, parasites and some viruses like HIV, influenza virus, RS virus and herpes simplex virus. MBL is located on chromosome 10q25, and there are seven distinct haplotypes influencing the stability of the protein and thereby its serum concentration. Up to 25% of the population has decreased levels of MBL. In earlier studies MBL-deficiency seems to be of clinical relevance when found in conjunction with other deficiencies of the immune system. This was first shown in children with combined MBL deficiency and IgGsubclass deficiencies.

Chemotherapy causes neutropenia and an increased chance for infections. Neth et al. enrolled 100 children receiving chemotherapy for malignancy. The main finding was that patients with MBL mutations had twice as many febrile neutropenic days compared to children with the wild-type genotype. There was no obvious relation between the frequency of Gram-positive bacteria, Gram-negative bacteria, or fungal infections and MBL-genotype. Peterslund et al. investigated 54 adult patients with a variety of hematological malignancies. The MBL level of patients with clinical severe infections was retrospectively compared with the MBL level of patients without infection. The MBL level was significantly lower in patients with clinically severe infection. Bergmann et al. prospectively studied 80 adult acute myeloid leukemia patients. 20% of these patients had low MBL levels. Low levels of MBL did not influence the incidence or duration of fever, or occurrence of septicemia or pneumonia. Kilpatrick et al. prospectively studied 54 adult patients with hematological malignancies. No significant relation was found between MBL deficiency and severity of infections. Two other retrospective studies were performed in allogenic transplant patients. Mullighan et al. studied 97 donor-recipient pairs undergoing allogenic bone-marrow transplant for a hematological malignancy. Of the 93 recipients 40.9% and of the 90 donors 42.2% carried an MBL2 coding mutation. Both MBL2 coding and promoter polymorphisms were associated with an increased risk of infection following transplantation, this was seen both for the donor and the recipient. The high-producing haplotype HYPA was associated with a markedly reduced risk of infection (both for recipient and donor). Rocha et al. studied gene polymorphisms and clinical first episodes of infection in 107 HLA-identical allogenic BMT for acute or chronic leukemia. MBL gene polymorphisms were not associated with more severe infections. The above 6 studies illustrate that MBL levels play a certain role in severity of infection in oncological patients treated with chemotherapy. However, there is controversy in the results, and so far only one prospective pediatric trial was performed. In chapter 3 of this thesis a prospective pilot study will be presented evaluating the level of MBL and genotyping MBL2 in relation with febrile neutropenia in a relatively small cohort of pediatric oncology patients.
2.3 The prognostic role of cytokines predicting the clinical course of infection

The acute-phase protein CRP has been widely used as a prognostic indicator for severity of febrile neutropenia but unfortunately there are disadvantages. CRP does not increase significantly until 24-48 hours after onset of inflammation and the serum concentration correlates with the grade of tissue damage and the activity of the underlying malignancy. Monitoring of serum cytokines may be used as an early diagnostic tool for bacterial infections before results of blood cultures are available. It has been well established that pro-inflammatory cytokines are released during infection. The cytokine response pattern in the first 24 hours after start of fever in neutropenic patients is important. Engervall et al were the first to describe this cytokine pattern. Gram-negative bacteremia in febrile neutropenic patients correlated with high levels of TNF-α, and IL-1ra (receptor antagonist) at the time of blood-culture; at 2-6 hrs after start of fever there were high serum levels of TNF-α, IL-1, IL-6 and IL-10. For Gram-positive bacteraemia no discriminative cytokine level was found. Steinmetz et al defined cut-off levels for IL-6 and IL-8 prior to the onset of fever in the neutropenic patient (the cut-off levels for predicting serious infection were IL-6 >15 pg/mL and IL-8 >130 pg/mL). De Bont et al could define a low-risk febrile neutropenic group on base of IL-6 and IL-8 levels at the start of the febrile neutropenic episode. Apart from the role of cytokines as predictors for severity of infection, the precursor protein of calcitonin, pro-calcitonin has been found to be an even more useful diagnostic inflammatory marker in febrile cancer patients than IL-6, IL-8 and CRP. Predicting bacteremic versus non-bacteremic infection pro-calcitonin was preferred to IL-8 (cut-off level 0.5 ng/mL, sensitivity 73% specificity 86%), but in predicting Gram-negative bacteremia IL-8 was superior.

IL-8 seems a reliable marker for severe infection such as Gram-negative bacteremia. IL-8 belongs to the family of chemokines (chemotactic cytokines). It is produced by various cell types, monocytes, lymphocytes and granulocytes. It seems unlikely that these are the main sources of IL-8 during febrile episodes and chemotherapy-induced neutropenia. Therefore other cell types must play a role in IL-8 production like endothelial cells, epithelial cells and fibroblasts.

Knowing that tissue-damage influences the cytokine pattern, a prognostic study was performed in pediatric oncology patients suspected of neutropenic enterocolitis, to gain insight in the pathology, immunology, cytokine levels, infectious causes and clinical follow-up of these patients. This will be presented in chapter 4.

3 Prevention of infection

3.1 Prevention of Gram-positive catheter related infections

Tunnelled central venous catheters have become convenient tools in the treatment of patients, especially in pediatric patients where venous access is poor and there is a need for prolonged administration of chemotherapy, blood products or total parenteral nutrition. The tunnelled
central venous catheters can be divided in total implantable devices (the subcutaneous port) and the non-fully implantable devices (most used are the Hickman and the Broviac catheter). These non-fully implantable devices can have single or multiple lumina. This is required when more agents need to be infused simultaneously like in bonemarrow-transplant patients. They are surgically inserted under sterile conditions by experienced personell. The distal end of the catheter is positioned in the superior caval vein or right atrium. Broviac and Hickman catheters are anchored by a Dacron cuff subcutaneously before exiting the skin. Fixation of the catheter usually occurs within 3-4 weeks after insertion. If possible the subcutaneous port is placed because the port is less visible, it preserves the body image of the patient, needs flushing less often than the Broviac or Hickman catheter and is less susceptible for infections. However, in patients undergoing allogenic bone-marrow transplantation and in patients at high-risk of tumor lysis (Burkitt lymphoma) the non-fully implantable device is preferred, because it can be predicted that multiple lumens are needed.

The reported rates of infection vary according to the intensity of the use of the catheter, the maintenance of the catheter and the underlying malignancy. The risk ranges from 1.4 to 2.8 infections per 1000 catheter days. Important in these reported rates are how catheter-related infection is defined and what diagnostic method was performed to prove the presence of a catheter-related infection.

### Table 1: Definitions of catheter-related infections (Mermel et al)

<table>
<thead>
<tr>
<th>Definitions</th>
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<tr>
<td><strong>Exit-site infection</strong></td>
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<tr>
<td>Evidence of cellulitis around the exit site, diagnosis can be made by inspection. If quantitative culturing in the laboratory is present then quantitative culturing of the skin or of the subcutaneous catheter segment may be helpful. An exit site infection may occur with or without a bloodstream infection.</td>
</tr>
<tr>
<td><strong>Tunnel-infection</strong></td>
</tr>
<tr>
<td>Evidence of cellulitis overlying the tunnel tract of subcutaneously tunneled catheters. There are signs of inflammation along the tunnel tract and there is tenderness to palpation over the tunnel tract</td>
</tr>
<tr>
<td><strong>Definite catheter-related infection</strong></td>
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| Isolation of the same organism from percutaneous blood culture and from one of the following:  
  a) exudate at the catheter exit site  
  b) a semiquantitative catheter segment culture (but this requires catheter removal)  
  c) quantitative blood culture with recovery of at least five fold higher colony count from blood obtained through the catheter than from a percutaneous blood culture. |
| **Suspected catheter-related infection** |
| a temporal succession of catheter flushing, onset of chills and fever and a positive blood culture, then highly suggestive of a catheter related infection.  
  a short time to positivity of the bloodculture is suggestive of a catheter related infection, this method makes use of continuous blood-culture monitoring and compares the differential time to positivity for qualitative cultures of blood samples drawn from the catheter and a peripheral vein. |
Commonly used definitions of intravascular catheter-related infections are divided in exit-site infection, tunnel infection, pocket infection and bloodstream infection. Most recent definitions have been summarized by Mermel et al. (Table 1).

The pathogens cultured from catheter-related infections are mainly Gram-positive organisms (CNS, Enterococci, St. aureus) in 70% of the cases, followed by Gram-negative organisms (Pseudomonas aeruginosa, Enterobacter spp, Acinetobacter spp, Serratia spp) in 15%, fungal organisms (Candida spp) in 8% and anaerobe micro-organisms in 7%.

Preventing these infections is of utmost importance. Obviously education and consistency in care are the mainstays of preventing infection.

As the most often cultured organisms are Gram-positive organisms, the role of antibiotic prophylaxis covering Gram-positive organisms at the time of placement of the catheter has been investigated. Until now this role has been found to be controversial. Until now this role has been found to be controversial.

In Chapter 5 a Cochrane systematic review is performed, to answer the question if antibiotic prophylaxis has to be given before insertion of the catheter. The use of catheter flush solutions has also been investigated in their role to prevent Gram-positive infections. In other groups of patients, mainly neonates it was proven that vancomycin-containing flush solutions decreased nosocomial Gram-positive bacteremia.

In oncology patients the results were conflicting therefore in the same systematic review in Chapter 5 all randomized controlled trials are presented assessing the effect of antibiotic flushing of the catheter.

3.2: Prevention of bacteremia during episodes of neutropenia using selective decontamination of the digestive tract (SDD)

As mentioned in the general introduction Bodey emphasized that neutropenia formed a risk-factor for infection already 35 years ago. Decreasing infections during neutropenia would therefore decrease morbidity and mortality due to infections.

In the early 70's, van der Waaij et al. developed a strategy to reduce the frequency of infections in the immunocompromised patients. By this strategy, named selective decontamination of the digestive tract (SDD), potentially pathogenic aerobic microorganisms are eliminated from the gastro-intestinal tract, without affecting the non-pathogenic anaerobic flora. SDD is based on a mechanism termed 'colonization resistance (CR)', in which the colonic anaerobic flora prevent colonization with new aerobic mechanisms. SDD is achieved by administration of oral partly absorbable and partly non-absorbable antibiotics, often in combination with anti-fungal prophylaxis. Currently trimethoprim/sulfamethoxazole and quinolones are the most widely used in this regard.

Many randomized trials have been performed, often double-blind placebo controlled but on small groups of patients. In those single trials SDD was found effective in reducing bacteremia and infection, but not in the prevention of fever or in the reduction of overall mortality. These data were presented in 2 systematic reviews. Resistance and the occurrence of Gram-negative bacteria resistant to quinolones or cotrimoxazole is a potential risk-factor of SDD. Another risk-
factor is the poor coverage of Gram-positive organisms with SDD. The addition of an oral, systemic antibiotic against Gram-positive cocci has shown to offer protection against streptococcal and other Gram-positive infections without reducing the overall infectious complication rate and without decreasing mortality due to infections. Despite the amount of studies involving SDD, there is no consensus whether SDD should be given and what types of antibiotics to use.

In Chapter 6, a systematic review was performed assessing all randomized trials looking at the efficacy of the different interventions for SDD, and the influence on death due to infection.

3.3 Prevention of varicella zoster infection by immunizing varicella IgG negative children with cancer

The requirements for successful vaccination vary according to the nature of the infecting organism. For extracellular organisms antibodies provide the most important adaptive mechanism of host defence, while for control of intracellular organisms an effective CD8+ T-lymphocyte response is essential. The ideal vaccination provides host defence at the point of entry of the infectious agent. Therefore mucosal immunity is of utmost importance as many organisms enter through the mucosa.

Live-attenuated viral vaccines are far more potent than killed viral vaccines. Probably because they elicit a greater number of relevant effector mechanisms, including cytotoxic CD8+ T-cells. Attenuation is achieved by growing the virus first in human cultured cells. Subsequently the virus is then adapted to growth in cells of a different species, until it grows only poorly in human cells. The virus acquires many mutations that allows growth in the cultured non-human cell but prevents growth in the human cell. It will therefore produce immunity but not disease. Attenuation may be achieved more rapidly and reliably using recombinant DNA techniques. The mutations created make it virtually impossible to revert to the wild-type virus.

Recommendations on vaccination during chemotherapy state that killed or inactivated vaccines do not represent a danger to the immunocompromised host, and as a general rule live attenuated vaccines should be administered at least 6 months after stopping chemotherapy. However, the immunogenic response to vaccinations is decreased during chemotherapy, but not zero, this enables us to vaccinate with certain vaccines. This makes it interesting in patients in who we can expect complications during or after the VZV infection and it is of special interest in area's where herd immunity is low. Prerequisites for vaccination are an adequate number of lymphocytes (>750 cells/mm³) an adequate number of neutrophils (>1000 cells/mm³) and no use of dexamethasone 14 days before the vaccination and one week after the vaccination.

As awareness of the morbidity and mortality due to varicella infection became established (see section 1.2.2), the interest in the live-attenuated varicella vaccine increased. This vaccine (the Oka-strain) was developed in Japan in the early 1970's and was approved by the Food and Drug Administration in 1995 for routine use in healthy persons older than one year of age who are susceptible to varicella. Japan, Korea and the majority of the states of the USA are including
varicella vaccination in their routine schedule. The goal of the Centers for Disease Control and Prevention of reaching more than 90% of children less than three years of age by 2010 seems achievable. A marked decline in the number of cases of varicella has been observed in the USA, and a non-significant trend towards less hospitalizations due to chickenpox.

In the USA 575 children with leukemia in remission were immunized in the Varicella Vaccine Collaborative Study. All children were in continuous remission for over 1 year or more. The varicella vaccine was found to be safe, immunogenic and effective. The major adverse reaction was a varicelliform rash, treated with oral acyclovir. The seroconversion to VZV occurred in 82% of vaccinees after 1 dose and in 95% after 2 doses. The benefits of varicella vaccination should be extended to oncology patients in an earlier phase of the treatment. One study administered varicella vaccine before the start of chemotherapy. Seroconversion was noted in 77% of 13 vaccinated children. Mild side-effects were observed in 12.5% of patients consisting of a varicelliform rash and fever. In this study it seemed safe to administer the vaccine, only mild side effects were seen, however, also in this study the onset of chemotherapy was delayed because of the vaccination.

Our aim was to study the efficacy of VZV vaccination in IgG-VZV negative pediatric oncology patients, without interrupting chemotherapy and introducing the vaccine in a relative early phase of the chemotherapy. Seroconversion early in their treatment will decrease the incidence of VZV infections and reduce the number of complications due to this infection. This ongoing study will be presented in Chapter 7.

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