Prediction and prevention of infectious complications in children with cancer
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Prediction and Prevention of infectious complications in children with cancer
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This thesis was prepared at the Department of Pediatric Oncology/Emma Children’s hospital, Academic Medical Center, University of Amsterdam, the Netherlands and at the Department of Pediatric Oncology/Baragwanath Hospital, University of Witwatersrand, Johannesburg, South Africa.

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Prediction and Prevention of infectious complications in children with cancer

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

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ten overstaan van een door het college voor promoties ingestelde

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Faculteit Geneeskunde
Aan mijn ouders
Umama, ubaba
Siyabonga
Prologue: the cover of the thesis

Ndbele people are offshoot of Nguni people of Kwazulu Natal, SOUTH AFRICA. They are well known for their artistic talent with regard to painted houses, colourful beadwork and the rings around their neck.

In early days they went through rough times but they survived and they show the world their bright future with their beads and colourful painted houses.

As a child living in South Africa I was fascinated by these people and the colours of their houses made the sun shine even brighter.

Now working as a pediatric oncologist in the Netherlands, I often think of these people, because a sunny bright view of life is often needed in the difficult field of pediatric oncology.

That is the reason why I chose this cover for this thesis to present the research leading to better control of infectious complications in the child with cancer just like the sun shining on the colours of the Ndbele people.

NKOSI SIKELEL' I-AFRIKA

Marianne
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Scope of the thesis:

Improvement has been made over the past 30 years achieving better survival in 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 still play a major role.

Prediction and prevention of infectious complications in children with cancer are the scope of this thesis. After a general introduction in chapter 1, chapter 2 will describe a retrospective cohort study in a pediatric oncology unit in South Africa focussing on causes of bacteremia and risk-factors for infection. Within the group of oncology patients it has become clear that some patients are more susceptible to infections than others, therefore chapter 3 presents a prospective cohort study on the role of mannan-binding lectin (MBL) answering the question if MBL deficiency in immuno-compromised children leads to a prolonged duration of neutropenia and to more severe infections. One of the most severe infectious complications in oncology patients is neutropenic enterocolitis. In Chapter 4a a case of neutropenic enterocolitis and typhlitis will be presented and in chapter 4b a prospective study will be presented including pediatric oncology patients with a clinical suspicion of neutropenic enterocolitis. The aim of the study is to gain insight into the pathogenetic mechanisms, and to identify clinical and inflammatory prognostic markers. It is known that this group of children is susceptible to infections therefore prevention of infections is even more important. One of the risk-factors for infection is the presence of a central venous catheter. Therefore in chapter 5 a Cochrane systematic review is presented on the use of prophylactic antibiotics to prevent Gram-positive bacteremia in tunnelled central venous catheters. On base of the available evidence a meta-analysis is done and a number needed to treat calculated to prevent these bacteremia’s. In oncology patients the use of selective decontamination of the digestive tract (SDD) is still a matter of debate. Therefore in Chapter 6 a systematic review was performed to assess the evidence for the effectiveness of SDD to decrease bacteremia and infection-related mortality during neutropenic episodes in oncology patients.

Where chapter 5 and 6 focus on prevention of bacterial infections, chapter 7 concentrates on prevention of varicella by immunizing IgG negative children with the live-attenuated varicella vaccine in an early stage of their disease. If protection can be achieved then fear for varicella infection and the possible complications will be clearly reduced. Insight in the different aspects of infectious complications in children with cancer will improve management and open directions for future trials, mentioned in Chapter 8.
Sanibonani ninjani
“hello how are you and your family”

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/mm³. This study showed an incidence of severe infections of 43 episodes per 100 admitted patients if the granulocyte count was below 500 cells/mm³, compared to <5 infectious episodes if the neutrophil count was >1500 cells/mm³. 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 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 $\geq 38.3^\circ$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. 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. The mortality with good medical management has been estimated at 20%. 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.

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. 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 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. 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 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 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 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. 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/mm$^3$ and the CRP was below 90 mg/L the risk for bacteremia was low (5%). A consistent trend has been shown in patients with a low neutrophil count (< 100 cells/mm$^3$) or no evidence of marrow recovery constituting the “high risk” group. If by day 4 of febrile neutropenia, the platelet count rises and the monocyte count is > 100 cells/mm$^3$, then this constitutes marrow recovery and patients can be defined as “low-risk”. The “low-risk prediction rule” in pediatric patients is not a clear-cut rule. Mullen et al 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 micro-organisms 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, and 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

Lectin pathway
Binding to carbohydrate on microbial surfaces

Alternative pathway
Binding to surfaces upon autoactivation

C1q
C1r
C1s

MBL
MASP-1
MASP-2
MASP-1?

Activation of C4 & C2

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. 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.

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.

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. 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, susceptibility to pneumococcal disease linked to defective production of antibody to the pneumococcal capsule. 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. 51-54. 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 IgG-subclass deficiencies.

Chemotherapy causes neutropenia and an increased chance for infections. Neth et al 55 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 57 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 58, 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 59 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 60 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 61 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 \(^{52,62}\). 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 \(^{62}\). Gram-negative bacteraemia in febrile neutropenic patients correlated with high levels of TNF-\(\alpha\), 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-\(\alpha\), 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) \(^{64}\). De Bont et al \(^{65}\) 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 \(^{62}\). 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 \(^{66}\).

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 \(^{65}\).

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 tunneled
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 bone marrow-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.\textsuperscript{67,68} 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\textsuperscript{69,70}. 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>
</tr>
</thead>
<tbody>
<tr>
<td>Exit-site infection</td>
</tr>
<tr>
<td>Tunnel-infection</td>
</tr>
</tbody>
</table>
| Definite catheter-related infection | 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 blood culture is suggestive of a catheter related infection, this method makes use of continuous blood-culture monitoring and compares the differential time to positivity for quantitative 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.\(^2\) (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\(^2\). 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\(^73\)–\(^76\). 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\(^77\)–\(^81\). In other groups of patients, mainly neonates it was proven that vancomycin-containing flush solutions decreased nosocomial Gram-positive bacteremia\(^82\). 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\(^4\) 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.\(^83\) 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\(^84\). 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\(^85\)–\(^86\). 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 (50). 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 whom 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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84. van der Waaij D, Berghuis-de Vries JM. Selective elimination of Enterobacteriaceae species from the digestive tract in mice and monkeys. J.Hyg.(Lond) 1974; 72:205-11.


Bacteremia in a pediatric oncology unit in South Africa

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Abstract

Objectives: To identify the morbidity and mortality due to infections in a South African pediatric oncology unit, and to identify risk factors associated with first bacteremic episodes in this unit.

Procedure: A retrospective cohort study was done in a large regional referral pediatric cancer center from 1991-1995, of all consecutive patients with culture proven bacteremia. Eighty-three oncology patients were studied (median age 4.0 years) in whom a total of 200 episodes of bacteremia were recorded, of which 83 first bacteremic episodes.

Results: Of the 200 episodes 70% were caused by Gram-positive organisms, 20% by Gram-negative organisms and 10% by fungal organisms. Organisms associated with high mortality were Gram-negative organisms (Acinetobacter spp., Pseudomonas aeruginosa, Klebsiella species), and fungal organisms, (Candida parapsilosis). Seventeen out of 200 episodes ended in death of the patient. In 59% of patients Hickman catheters were in situ. The mean incidence of catheter related bacteremia's was 3.3 episodes per 1000 catheter days. Seventy percent of first bacteremic episodes occurred within 50 days after placement of the catheter. The generalized estimation equations model revealed that more Gram-negative infections occurred in the presence of a Hickmancatheter (odds ratio 2.2, 95% CI 1.0-5.0). The presence of neutropenia and the use of parenteral nutrition were not associated with specific bacteremic patterns.

Conclusions: Including all bacteremic episodes in this cohort study a high incidence of fungal infections occurred of which 64% occurred with a Hickmancatheter in situ. Candida parapsilosis had a higher incidence than reported in other centres. Secondly looking at first bacteremic episodes a high incidence of Gram-negative infections was observed especially in the presence of a Hickmancatheter.
Introduction

Although the management of the child with cancer has improved dramatically over the past 20 years, significant morbidity and mortality from infectious complications are still seen (1,2,3). A number of risk factors are important in contributing to infectious complications. The major risk factor leading to infection in patients with cancer is therapy-induced neutropenia as defined by an absolute neutrophil count (ANC) less than 500 cells /mm³.

A subpopulation with the highest risk of infection are those who experience more profound (ANC <100 cells/mm³) and prolonged (>7 days) neutropenia. Intensifying chemotherapeutic protocols, allogenic and autologous bone-marrow transplants have led to longer periods of profound neutropenia.(3,4,5)

The second important risk factor within the group of oncology patients is the increased use of indwelling central venous catheters (ICVC). The mean incidence of ICVC-related bloodstream infections in hospitalized pediatric patients is 2.4 episodes per 1000 catheter days (6,7). Totally implantable devices are associated with a lower risk of infection, these are reported to have a 20 fold lower risk of infection than external ICVC’s (6). Microorganisms most commonly causing ICVC related bacteremia’s are coagulase-negative staphylococci, Staphylococcus aureus and Candida sp. Gram negative bacilli are considered less frequent and generally related to a hospital environment (7).

The last risk factor mentioned is the use of intravenous hyperalimentation. The group receiving parenteral nutrition is at greater risk for developing infections for several reasons. These are post surgical patients, critically ill patients with bowel pathology, patients with a poor nutritional status and patients who receive intensive chemotherapy. In 1985 Wolfe (8) examined infectious complications of the use of parenteral nutrition. Organisms isolated were Staphylococci , fungi and Gram negative bacilli.

Limited data are available on infectious complications in pediatric oncology patients in countries, where most patients come from a rural area. Most children are admitted in an advanced stage of their disease. The poor housing circumstances, the long travel distances and the severity of the disease might influence the risk of infection.

The purpose of this study was to investigate:

a) Causes of bacteremia and characteristics of children with a malignant disorder admitted to a South African pediatric oncology unit.

b) To record the association between central venous catheters, neutropenia, hyperalimentation, and the different groups of organisms (Gram-negatives, Gram-positives and fungal organisms.

c) To look at outcome of the recorded bacteremia’s, ie.

1) the duration of the infection,
2) the duration to initiating the correct antibiotic therapy,
3) the mortality.
Patients and methods

A retrospective analysis was performed in the pediatric oncology unit of Baragwanath Hospital (Johannesburg) from 1-1-1991 to 1-1-1995. Baragwanath hospital is a large teaching hospital that provides primary, secondary and tertiary care facilities for the pediatric population of the greater Soweto (Johannesburg) and acts as a referral center for a large part of Southern Transvaal, Northern Natal, Orange Free state and several South African countries. In the oncology unit about 200 new patients are seen on a yearly basis, mainly black children, with advanced stage cancer. Of all new patients admitted 20% present with leukemia, 10% with lymphoma's and 70% with solid tumors. They are admitted for long periods of time (the average hospital stay per patient is 83 days) because of their poor housing circumstances, long distances to home and poor transport facilities.

In this study all data on positive bloodcultures were collected from the S.A. Institute of Medical Research over this time period (1991-1995) after which the medical records were studied. The inclusion criteria for the study were: Newly diagnosed oncology patients admitted to Baragwanath Hospital who received chemotherapy and/or radiotherapy/surgery. Patients who only received radiotherapy/surgery were excluded from the study.

The information extracted from all study subjects included: age, sex, underlying diagnosis, catheter insertion date, date of catheter removal and cause of catheter removal. For all bacteremic episodes recorded the following data were extracted: Date of the infection, type of infection, presence or absence of neutropenia, pathogen causing the infection, the initial therapeutic regimen, and after the pathogen susceptibility was available time to start appropriate antibiotic therapy, and outcome of the bacteremic episode.

Definitions (9)

Bacteremia/ Fungemia- Presence of at least one single positive blood culture, in presence of fever with no other obvious focus of infection except for the indwelling catheter.

Fever- Rectal Temperature > 38.5 C. on 2 separate occasions over a 12 hour period.

Granulocytopenia- Neutrophils < 500/mm³, severe neutropenia < 100/mm³.

Outcome

The outcome of each bacteremic episode was categorized into 4 groups. The outcome data were retrospectively extracted from the records by MvdW (author)

1) The patient had fever, but no other signs of sepsis (no increased heartrate, no drop in blood pressure) and recovered without antibiotic therapy.
2) The patient was clinically ill (lethargic + one other sign of sepsis) and responded to antibiotic therapy within 48 hours (Fever improved and clinically better).
3) The patient was clinically ill, and recovery was delayed (with clinical signs of infection persisting) necessitating a change in antibiotic therapy.
4) The bacteremic episode ended in death of the patient.

Quality of care:
Baragwanath hospital runs 2 separate oncology wards for pediatric patients. There are 3 specialized consultants, 1 fellow in hematology, and 2 registrars. There are 20 beds in each unit, nursing-care consists of 4 qualified nurses per shift, 2 nurse-aids and 2 junior nurses. All patients are treated according to standard European and North-American protocols.

The standard protocol for oncological patients with neutropenic fever existed of starting iv. antibiotics, i.e. Imipenem and Amikacin. If no clinical response within 48 hours Vancomycin was added. After 5 days of therapy and no clinical response Amphotericin B i.v. was added. During neutropenia no patients were isolated, and no extra dietary measures were taken.

Statistics
We used standard statistical techniques to describe the characteristics of patients and their infections. For patients with a Hickman-catheter developing a first bacterial infection, we constructed a Kaplan-Meier curve to display the length of time since placement of the catheter till the first bacteremic episode.

We performed a logistic regression analysis to examine whether more Gram-negative infections occurred in the presence of a Hickman catheter or neutropenia. For these analyses we excluded fungal infections because of their low frequency, and analysed up to three bacteremic episodes per patient. We used generalized estimating equations models to adjust for the correlation between multiple episodes within the same patient. (28,29) The robust estimate of the standard errors was used to calculate 95% confidence intervals around odds ratio's. These odds ratio's express the relation between the presence of a Hickman line or neutropenia (independent variables) and the occurrence of a Gram negative infection (dependent variable).

The clinical outcome in relation to the type of organism (Gram positive or Gram negative) causing the first bacteremic episode was analysed using the Chi-square test. A p-value less than 0.05 was considered statistical significant.

All statistical analyses were done using SPSS software, except for the general estimation equations models which were performed using SAS 6.12 (GENMOD procedure).

Results
During the study-period a total of 2302 bloodcultures were taken in pediatric oncology patients of these 331 bloodcultures were positive (14.3%). Indication for bloodcultures were fever during neutropenia, repeat bloodcultures and routine bloodcultures on the indwelling catheters done 2x a week. Excluding the repeat bloodcultures 200 separate episodes of bacteremia and/or fungemia were recorded in 83 patients (51 males, 32 females) The median age of the children
was 4.0 years (range 3 months -14 years) 45.8% had hematological malignancies, 54.2% solid tumors. The median number of bacteremic episodes per patient was 2.0. The various patient characteristics are shown in Table I.

**Characteristics of infections**

Of the 200 organisms recorded 70% were Gram-positive organisms, of these Gram-positive organisms the organisms that were defined as low virulence were: *Bacillus spp. Corynebact. spp. S. epidermidis and S. viridans.* 20% were Gram negative, and 10% fungal organisms. 83 episodes were recorded as first infection. The distribution of the organisms on first infections was 79% Gram-positive organisms, 20% Gram-negative organisms and 1% fungal organisms. The organisms are shown in Table II.

### Table I: Patient characteristics

<table>
<thead>
<tr>
<th>Patient factor</th>
<th>3months-14 years</th>
<th>median 4.0 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>61.4%</td>
<td>38.5%</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Tumor type</td>
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<td></td>
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<tr>
<td>Solid</td>
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<td></td>
</tr>
<tr>
<td>Hematological</td>
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<td></td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
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<tr>
<td>Solid tumor types</td>
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<tr>
<td>Neuroblastoma</td>
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</tr>
<tr>
<td>Nephroblastoma</td>
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<tr>
<td>Rhabdomyosarcoma</td>
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<td></td>
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<tr>
<td>Retinoblastoma</td>
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<td>Brain tumors</td>
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<tr>
<td>Other</td>
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<td>Hematological Malignancies</td>
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</tr>
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<td>ANLL</td>
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<td>Hodgkin’s lymphoma</td>
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</tr>
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<td>CML</td>
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<td></td>
</tr>
<tr>
<td>Hickman catheter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td>59.0%</td>
</tr>
<tr>
<td>No</td>
<td>34</td>
<td>41.0%</td>
</tr>
<tr>
<td>Bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>No fever</td>
<td>85</td>
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</tr>
<tr>
<td>Duration fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before positive culture</td>
<td>2 days (range 0-32 days)</td>
<td></td>
</tr>
<tr>
<td>After positive culture</td>
<td>3 days (range 0-53 days)</td>
<td></td>
</tr>
</tbody>
</table>

Table II: The absolute number of infections is recorded for Gram-positive, Gram-negative and fungal organisms, with in brackets the absolute number of first infections.

<table>
<thead>
<tr>
<th>GRAM-POSITIVES</th>
<th>Total (First)</th>
<th>GRAM-NEGATIVES</th>
<th>total (First)</th>
<th>FUNGAL</th>
<th>total (First)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>61 (27)</td>
<td>Acinetobacter spp.</td>
<td>9 (2)</td>
<td>C. parapsilosis</td>
<td>8 (0)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>27 (17)</td>
<td>Klebsiella spp.</td>
<td>6 (5)</td>
<td>C. albicans</td>
<td>7 (0)</td>
</tr>
<tr>
<td>Corynebacterium.</td>
<td>16 (7)</td>
<td>Escherichia coli</td>
<td>5 (2)</td>
<td>other Candida</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Viridans streptococci</td>
<td>13 (9)</td>
<td>P. aeruginosa</td>
<td>5 (2)</td>
<td>other fungi</td>
<td>2 (0)</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>13 (3)</td>
<td>Enterobacter spp.</td>
<td>5 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>5 (1)</td>
<td>H. influenzae</td>
<td>3 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>4 (2)</td>
<td>Salmonella</td>
<td>3 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>Other</td>
<td>4</td>
<td>Anaerobic</td>
<td>1</td>
</tr>
</tbody>
</table>

Risk factors:

1. **Indwelling central venous catheters:** Hickman catheters were used in 49 patients (59%) (61.2% solid tumours, 38.8% haematological malignancies). One hundred and ten bacteremic episodes were recorded in presence of the Hickman catheter.

In the children with solid tumors 60 bacteremic episodes and in the children with hematological malignancies 50 bacteremic episodes were recorded. With a Hickman line in situ 37 first bacteremic episodes were recorded. There were 12 patients who experienced their first bacteremic episode before placement of the catheter, therefore their first infection in the lifetime of the catheter was not recorded as their first bacteremic episode. The distribution of infections showed 70% Gram-positive organisms, 27% Gram-negative organisms and 3% fungal organisms.

The mean incidence of catheter related infections was 3.3 episodes per 1000 catheter days.

The median number of days the catheter was in situ was 75 days (range 1-238 days). The median number of days before the first bacteremic episode was 32 days (range 1-199 days).

The cumulative incidence curve on first infections shows that 70% of catheter infections occur within 50 days of placing the catheter (see Figure I)

![FIG 1: Number of days that the Hickman-catheter was inserted plotted against the cumulative incidence of first bacteremic episodes](image-url)
Table III: The bacteremic episode is stratified, recording the presence or absence of the Hickman-catheter and stating the absolute number of Gram-positive Gram-negative and fungal infections.

<table>
<thead>
<tr>
<th>Bacteremic episode</th>
<th>Gram-neg infections</th>
<th>Gram-pos infections</th>
<th>Fungal infections</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hmanline-no</td>
<td>7</td>
<td>39</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>26</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>17</strong></td>
<td><strong>65</strong></td>
<td><strong>1</strong></td>
<td><strong>83</strong></td>
</tr>
<tr>
<td>2 Hmanline-no</td>
<td>3</td>
<td>20</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>21</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
<td><strong>41</strong></td>
<td><strong>7</strong></td>
<td><strong>58</strong></td>
</tr>
<tr>
<td>3 Hmanline-no</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
<td><strong>19</strong></td>
<td><strong>9</strong></td>
<td><strong>36</strong></td>
</tr>
<tr>
<td>4 Hmanline-no</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Yes</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>12</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>4</strong></td>
<td><strong>10</strong></td>
<td><strong>1</strong></td>
<td><strong>15</strong></td>
</tr>
<tr>
<td>5,6,7 Hman line yes</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1</strong></td>
<td><strong>6</strong></td>
<td><strong>1</strong></td>
<td><strong>8</strong></td>
</tr>
</tbody>
</table>

Of these first infections related to the catheter 27% were Gram-negative organisms, 70.3% Gram-positive organisms and 2.7% fungal organisms.

The generalized estimation equations model showed that more Gram-negative infections occurred in the presence of a Hickman catheter (odds ratio 2.2: 95% CI 1.0-5.0), comparing this to no Hickman catheter present, although it must be realized that the number of bacteremic episodes is small and the confidence interval found wide. Of the Gram negative infections 9 (=22.5%) were due to *Acinetobacter* species of which 5 were *A. Baumanii* and 4 were *A. Iwoffi*. *A. Baumanii* was cause of high morbidity. It is well known that *A. Baumanii* is an important nosocomial pathogen and can lead to serious infection previously reported in neonatal units and intensive care units (19). Of the 5 *A. Baumanii* infections 3 ended in death of the patient, most likely due to delay in starting adequate antibiotic therapy. Table III does show a pattern where more fungal episodes are recorded as 2nd and 3rd bacteremic episode, this might be related to the prolonged hospital stay of these patients.

2. **Neutropenia:** In 86 (43%) bacteremic episodes children were neutropenic. Of these 52 occurred with severe neutropenia (26% of the total group). In the majority of bacteremic episodes (91.5%) the neutropenia resolved within 14 days. In 8 cases there was prolonged neutropenia. Organisms isolated included *Klebsiella* species, *Candida parapsilosis*, *Staphylococcus aureus*, and resistant *Acinetobacter*. Of the 83 first bacteremic episodes 38 were neutropenic (45.7%) and of these 20 (24%) had severe neutropenia. Organisms cultured during these severely neutropenic episodes showed 17 Gram-positive organisms (85%) and 3 Gram-negative organisms (15%). Neutropenia was not associated with a higher risk for Gram-negative infections. The odds ratio for developing Gram-negative infections was 0.7 (95% CI 0.3-1.6) in the presence of neutropenia, comparing this with no neutropenia present.

3. **Parenteral Nutrition:** Only 8 patients (9.6 %) received intravenous alimentation. Statistical
analyses were not done because of the small numbers. All children experienced bacteremic episodes. Organisms cultured included Gram-negatives (*Pseudomonas*) and fungal infections (*Candida* species).

**Outcome of bacteremic episodes:**

Of the 200 episodes recorded, 40 had outcome 1 (not clinically ill), 95 episodes with outcome 2 (ill and rapid recovery), 48 episodes with outcome 3 (ill and slow recovery) and 17 episodes ended in death of the child. The group of organisms associated with high mortality were Gram-negative infections, mainly *Acinetobacter* species, *Pseudomonas aeruginosa*, *Klebsiella* species, and fungal organisms, mainly *Candida parapsilosis*.

In Table IV first bacteremic episodes and outcome are presented. The clinical outcome following Gram-positive infections was more favorable than after Gram-negative episodes (p=0.01 chi-square test).

**Table IV:** The outcome of first bacteremic episodes is recorded in 4 categories (1-4) Outcome 1: not clinically ill, rapid recovery, Outcome 2: clinically ill, good response to antibiotic treatment, Outcome 3: clinically ill, poor response, change of antibiotic management, Outcome 4: the bacteremic episode leads to the death of the patient. The percentage Gram-positives, Gram-negatives, and fungal organisms is presented within each outcome category.

<table>
<thead>
<tr>
<th>Organism/outcome</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-pos</td>
<td>14 (21.6%)</td>
<td>33 (50.7%)</td>
<td>15 (23.1%)</td>
<td>3 (4.6%)</td>
<td>65</td>
</tr>
<tr>
<td>Gram-neg</td>
<td>1 (5.9%)</td>
<td>7 (41.2%)</td>
<td>4 (23.5%)</td>
<td>5 (29.4%)</td>
<td>17</td>
</tr>
<tr>
<td>Fungal</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>40</td>
<td>20</td>
<td>8</td>
<td>83</td>
</tr>
</tbody>
</table>

**Use of Antimicrobial Therapy:**

Of the 200 bacteremic episodes 159 were treated with antibiotic therapy. The 41 episodes where antimicrobial therapy was not started causative organisms were either of low virulence, or the patient had recovered when the blood culture result became available. In 100 episodes (62.8%) appropriate antimicrobial therapy was started immediately and the organism cultured was subsequently found to be sensitive to the initial empiric antibiotic therapy.

In 59 episodes there was a delay of one to seven days before appropriate therapy was started. Organisms associated with delayed appropriate therapy and a poor outcome included fungal organisms (n=10) and highly resistant organisms *Acinetobacter* species (n=5).

Of all Hickman catheters 21 were removed because of sepsis. 12 (12%) of 100 appropriately treated episodes ended in catheter removal, compared to 9 (15%) of 59 episodes where delayed appropriate therapy was started. (All these 9 episodes were fungal organisms and resistant *Acinetobacter* sp.)
Discussion

In this study the causes of bacteremia in a South African Pediatric Oncology centre were analysed. The prevalence of bacteremia recorded over the study period in this unit was 14.3 %. The prevalence reported in the literature ranges from 5-67% (10). The wide range reported is most likely due to differing criteria for taking blood cultures. In most centres patients are treated as out-patients and are only admitted when complications arise.

At Baragwanath hospital most children require a prolonged hospital stay, (mean 83 days). Children who would be treated as outpatients in other centres are inpatients in this center. Blood cultures are taken 2x a week routinely in all patients with a central venous catheter in situ. The prevalence of bacteremia in general pediatrics at Baragwanath hospital over the same time period was 10 %, (data from the South African Institute of Medical Research) The prevalence, causes and risk-factors for first infection were comparable to previous studies in oncology centers in developed countries. (2,4,11). Different was the high mortality seen from fungal infections (21%), mainly Candida parapsilosis and the high mortality of Gram-negative infections (20%) where resistant organisms like Acinetobacter species, cause high morbidity and mortality.(4)

Fungemia, a well known serious infection in the immunocompromised host (12) is of major concern in our unit. Very little is known about rates and risks of candidal infections in developing countries (13). The major risk-factors for fungemia are the presence of central venous catheters and neutropenia (14). In our study 64 % of fungal infections occurred in patients with Hickman catheters. Of the fungal infections in this unit 42 % were caused by Candida parapsilosis. This rate is higher than that reported in other centers (3-27% of all fungemias) (15) Possible causes for this increased incidence might be the more crowded conditions in these hospitals, poor infection-control, the lack of use of selective gut decontamination (16) and frequent use of broad spectrum antibiotics. The latter two causes might consequently lead to high rates of yeast colonization in hospitalized children (17) which can be transmitted indirectly between patients via contaminated hospital surfaces or (para) medical personnel (18).

In this study 64% of fungal infections had a poor outcome of which 21% ended in death. Delay in starting appropriate therapy might have contributed to the poor outcome. The higher incidence of fungal infections might warrant the early start of antifungal therapy (that is after 48 hours) if there is no response to antibiotic therapy with negative blood cultures.

The second notable finding was the difference in pattern of organisms in presence of the Hickman catheter. Not Gram-positive organisms but Gram-negative organisms were significantly more present, and mainly the resistant Gram-negative organisms led to a poor outcome. In review studies (4) it has been shown that the pattern of Gram-negative infections is changing. Klebsiella, Enterobacter and Serratia spp are shown to increase, E Coli decreases. In our unit the incidence of highly resistant Acinetobacter species increased.

A. Baumanii was cause of high morbidity. It is well known that A. Baumanii is an important
nosocomial pathogen and can lead to serious infection previously reported in neonatal units and intensive care units (19). Of the 5 A. Baumanii infections 3 ended in death of the patient, most likely due to delay in starting adequate antibiotic therapy. Possibly due to prolonged hospitalization in this unit more A. Baumanii are being cultured. Earlier catheter removal might improve the outcome of the patient (20).

A third important finding is that most first bacteremic episodes in presence of a Hickman catheter develop within the first 2 months after placement of the catheter. The Kaplan-Meier graph shows that 70% of first bacteremic episodes occur within 50 days after placement. Studies have been presented in the literature that confirm this finding (21,22) which has led to small intervention studies where vancomycin prophylaxis is given when placing the catheter (23,24) or the catheter is flushed with vancomycin the first 30 days after placement in stead of heparin, (25,26,27). The studies have controversial results, and neither method was implemented in the above study done. Implementing these interventions will possibly decrease the number of Gram positive infections but will not alter the rate of Gram-negative infections that play such an important role in this unit. It is however a point of concern and needs further attention.

The limitations of this retrospective cohort study are realized. It is however of importance to gain insight in infectious complications in pediatric oncology patients where most patients present in a late stage of their disease and come from a rural area. By knowing which pathogens play a role during treatment of this group of patients allows us to consider intervention studies concentrating on reducing Gram-negative and fungal infections, which can ultimately lead to a better outcome of the patient.

Acknowledgements:
The authors gratefully thank Prof. Dr. P. A. Voûte for his professional advice in this work and we also thank Dr. S. Heisterkamp and Dr. H. Reitsma for their advice and help with the statistical part of the paper.

References:
Mannan-Binding Lectin (MBL) serum levels in pediatric oncology patients: a pilot-study

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The Netherlands

Study in progress
Abstract

The febrile neutropenic patient with cancer forms a heterogenous group. A small group of these patients will be at high risk for serious infectious complications. It is thought that Mannan-Binding Lectin (MBL) deficiency as part of the innate immunity could lead to serious infections in this group of patients.

A prospective cohort study was performed in a single pediatric oncology unit genotyping the patients who were expected to become neutropenic. Data were collected on day 1, 3 and 5 of the febrile neutropenic episode correlating the MBL-genotype and the MBL serum level to the severity of the infection.

We included 40 children with cancer and of these 24 could be followed during a neutropenic febrile episode. The incidence of MBL exon-1 gene mutations and MBL serum level deficiency (<800 µg/L) was found to be 32.5%. In the deficient group there was no relation found with severity of infection, but there was a trend towards a prolonged duration of neutropenia in the deficient group (p=0.07). A possible explanation is that 70% of patients were severely neutropenic (<100 cell/µL) and 22.5% of patients were relapse patients. Because of the severe neutropenia the effector function of MBL might be severely compromised. Extending the cohort of patients might clarify the significance of the prolonged duration of neutropenia. This will answer the question in which group of patients MBL substitution will be of benefit.
Introduction

Although the treatment of pediatric oncology patients has dramatically improved over the past 20 years, infections still play a major role in morbidity and mortality\textsuperscript{1,3}. Although many patients experience severe and prolonged neutropenia not all patients suffer from the same complications due to infection during a neutropenic episode. The reasons for this are not clear but low levels of MBL might play a role.

MBL is a serum protein produced in the liver, and plays a critical role in the innate immune response. It is a collagenous lectin with two roles in host defense\textsuperscript{4}. First, it binds to sugars, in particular N-acetylgalactosamine and mannose, on the surface of many different micro-organisms and facilitates their opsonization.

Secondly MBL activates the classical route of the complement system via the so-called lectin route by means of two MBL-associated serine proteases (MASP's). The result is direct complement mediated lysis and opsonization followed by uptake by phagocytes.

A single functional gene (\textit{MBL2}) at chromosome 10q25 codes for human MBL\textsuperscript{5}. This \textit{MBL} gene consists of four exons. MBL deficiency is due to structural gene mutations in exon-1 i.e. at codon 52 (D variant), codon 54 (B variant) and codon 57 (C variant). The A variant represents the "wild type" or normal MBL. In addition to the structural gene mutations there are several polymorphisms within the promoter region of the \textit{MBL} gene. The four promoter haplotypes most commonly found are LXP, LYP, LYQ and HYP. The gene mutations are in linkage with the promoter polymorphisms and every individual will express two of the seven possible haplotypes-HYPA, LYPQ, LYPA, LYPB, LYQC and HYPD\textsuperscript{6,9}. Serum levels are dominantly influenced by the 3 mutations in exon 1 and modulated by the recessive promoter region polymorphisms.

The concentration of MBL possessed by an individual is genetically determined by the two haplotypes inherited from the parents. MBL deficiency is thought to be clinically important in patients with co-existing immune-defects, including primary and secondary immune deficiencies. Deficiency of MBL was first identified in children with an opsonization defect\textsuperscript{10}. Subsequent studies focussed on the role of MBL deficiency in relation with severity of infection. To date 3 prospective studies have been performed in oncology patients, of which one was performed in children\textsuperscript{17} and 2 studies only included adult oncology patients\textsuperscript{12,13}. Three retrospective studies were performed, 2 in allogenic transplant patients\textsuperscript{14,15} and one study including patients with various hematological malignancies\textsuperscript{16} (Table 1). However, there is controversy about the results of these studies. In the study of Neth \textit{et al}\textsuperscript{11} MBL-deficient pediatric oncology patients were shown to experience longer episodes of febrile neutropenia. Peterslund \textit{et al}\textsuperscript{16} and Mullighan \textit{et al}\textsuperscript{14} found more severe infections in a group of adult oncology patients. Possibilities are now available to start replacement MBL, with a plasma-derived product, the first clinical applications have shown no adverse effects, no antibody response to MBL, and a biological half life of 5-7 days\textsuperscript{17}. A prospective study needs to gain insight in the patients who will benefit the most from replacement therapy with MBL.
Table 1: Literature summary

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patient</th>
<th>malignancy</th>
<th>Duration FN</th>
<th>Freq. Infections</th>
<th>Severity Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neth'1</td>
<td>Prospect</td>
<td>Child N=100</td>
<td>All Malignancies</td>
<td>Sign. longer duration</td>
<td>=</td>
</tr>
<tr>
<td>Bergmann'2</td>
<td>Prospect</td>
<td>Adult N=80</td>
<td>ANLL</td>
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</tr>
<tr>
<td>Kilpatrick'3</td>
<td>Prospect</td>
<td>Adult N=128</td>
<td>Hematologic Malignancies</td>
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<td>=</td>
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<tr>
<td>Peterslund'4</td>
<td>Retrospect</td>
<td>Adult N=54</td>
<td>Hematologic Malignancies</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Mullighan'4</td>
<td>Retrospect</td>
<td>Adult N=97</td>
<td>Allogenic BMT</td>
<td>=</td>
<td>=</td>
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<tr>
<td>Rocha'5</td>
<td>Retrospect</td>
<td>Adult N=107</td>
<td>Allogenic BMT</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

Patients and methods

Study design

From February 2003 until November 2003 we included pediatric oncology patients admitted to the pediatric oncology unit of the Emma Children’s Hospital in Amsterdam. Patients between one and 18 years of age, who were expected to become neutropenic on base of the chemotherapy given, were considered for inclusion. Patients admitted for an autologous bone-marrow transplant were excluded. The study protocol was approved by the local ethics committee. Informed consent from parents and children (>12 years) was obtained.

Procedures

At the start of chemotherapy a blood-sample was taken for MBL-genotyping and the serum level of MBL was determined. The patient was followed during episodes of febrile neutropenia. Both clinical parameters and laboratory investigations were done on day 1, 3 and 5, and MBL-serum levels were determined on these days. The clinical outcome-parameters included were duration of fever, duration of neutropenia, signs of septicemia, intensive-care admission, and mortality due to infection. The routine laboratory investigations consisted of a full blood count, CRP, liver enzymes and (blood)-cultures. Other investigations such as chest X-ray were included when clinically indicated. Febrile neutropenia was defined as a single temperature of greater than 38.5°C, and neutropenia was defined as an absolute neutrophil count < 500 cells /mm³. Bacteremia was defined as the presence of clinical signs and symptoms of infection together with the isolation of bacterial pathogens from the blood.
Clinical parameters
Vital parameters (respiration rate, heart rate, blood pressure) and clinical signs were recorded on a case record form. Clinical signs scored included symptoms of illness (signs of sepsis, signs of airway problems, signs of abdominal complaints) and the fever pattern. All clinical signs were scored using the common toxicity criteria (CTC). The investigators who scored the clinical signs were blinded to the MBL genotype and serum level of the patient.
Laboratory investigations
Full blood count, CRP, liver enzymes, creatinine, and blood cultures were all performed, according to standard laboratory procedures.

MBL genotyping
Codon $^{52}$Cys, $^{54}$Asp and $^{57}$Glu on exon 1 were genotyped using the polymerase chain reaction and sequence-specific primers as previously described. In this technique alleles with each of the coding polymorphisms are directly amplified using forward and reverse allele-specific primers. Genotyping was performed independently of clinical data collection.

MBL serum level
Serum MBL concentrations were determined in a solid phase ELISA with mannan coated to the solid phase and a monoclonal antibody (biotinylated mouse-anti-MBL IgG 5E12, 10 µg/mL). Briefly, microtiter plates were coated with 100µL (10 µg/mL) mannan in 0.1M NaHCO$_3$, pH 9.6 overnight at room temperature. The microtiter plates were washed 5 times with H$_2$O. Serum samples and MBL standards (standard serum, 1.5 µg/mL MBL) were diluted in TTG/Ca (20 mM Tris pH 7.4/150 mM NaCl/0.02% TWEEN-20/0.2% gelatin/10mM CaCl$_2$), with 10 U/mL heparin for testing serum, and incubated shaking at room temperature for 1 hour. After washing, the plates were incubated for 1 hour with biotinylated MAb5E12 in TTG/Ca and washed 5 times with H$_2$O and were then incubated at room temperature for 30 minutes with streptavidin pHRP 1:10000 in TBS/Ca/2% (20mM Tris pH 7.4/150 mM NaCl /10 mM CaCl$_2$ /2% milk). After washing, color was developed using tetramethyl-3,3,5,5-benzidin (TBM)/H$_2$O$_2$ in 0.1 M NaAc pH 5.5 and stopped with 2M H$_2$SO$_4$, A BioAssay Reader, HTS 7000 plus (Perkin Elmer) measured spectrophotometric absorbances at 405 nm. The cut-off point for MBL deficiency was a plasma concentration of 800 µg/L (i.e. above the lower limit of the 95% CI from 200 control individuals without exon-1 mutations).

Statistics
Patients were classified according to the genotype of MBL and divided in a MBL-sufficient (>800 µg/L) and a MBL-insufficient group (<800 µg/L) The number of days of fever, duration of neutropenia and outcome of the febrile neutropenic episode in patients with structural gene mutations were compared with patients without structural gene mutations by means of the
Mann-Whitney U test, or the chi-square test where appropriate. If the number was less than five in one of the cells we performed a Fisher’s exact test.

Changes in concentrations of MBL in serum during febrile neutropenic episodes were analysed by the paired t-test.

To evaluate the prognostic value of the MBL serum level we used both the cut-off mentioned in the literature (above 800 µg/L) and also an optimal cut-off point selected from our own data, obtained with a receiver-operator characteristic curve (ROC). We used SPSS 11.0 computer software.

### Table 2: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total Group N=40</th>
<th>Not febrile group N=16 (Group1)</th>
<th>Febrile neutropenia N=24 (Group 2)</th>
<th>p-value between Group 1 and 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age median years</td>
<td>8.7 years (1.8-16.7)</td>
<td>9.7 years (1.8-16.7)</td>
<td>8.1 years (1.9-16.6)</td>
<td>0.22</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>27 (67.5%)</td>
<td>13 (81.3%)</td>
<td>14 (58.3%)</td>
<td>0.12</td>
</tr>
<tr>
<td>female</td>
<td>13 (32.5%)</td>
<td>3 (18.8%)</td>
<td>10 (41.7%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hematological</td>
<td>17 (42.5%)</td>
<td>6 (37.5%)</td>
<td>11 (45.8%)</td>
<td>0.42</td>
</tr>
<tr>
<td>solid</td>
<td>23 (57.5%)</td>
<td>10 (62.5%)</td>
<td>13 (54.2%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Relapse patients</td>
<td>9 (22.5%)</td>
<td>3 (18.7%)</td>
<td>6 (25.0%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Gene mutation</td>
<td></td>
<td></td>
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<td>0.66</td>
</tr>
<tr>
<td>AA</td>
<td>21 (52.5%)</td>
<td>8 (50.0%)</td>
<td>13 (54.2%)</td>
<td>0.66</td>
</tr>
<tr>
<td>AO</td>
<td>10 (25.0%)</td>
<td>3 (18.8%)</td>
<td>7 (29.0%)</td>
<td>0.66</td>
</tr>
<tr>
<td>OO</td>
<td>3 (7.5%)</td>
<td>2 (12.5%)</td>
<td>1 (4.1%)</td>
<td>0.66</td>
</tr>
<tr>
<td>missing</td>
<td>6 (15.0%)</td>
<td>3 (12.5%)</td>
<td>3 (12.5%)</td>
<td>0.66</td>
</tr>
<tr>
<td>MBL level Day 0*</td>
<td></td>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>&lt;800 µg/L</td>
<td>13 (32.5%)</td>
<td>6 (40.0%)</td>
<td>7 (29.2%)</td>
<td>0.36</td>
</tr>
<tr>
<td>&gt;800 µg/L</td>
<td>26 (65.0%)</td>
<td>9 (60.0%)</td>
<td>17 (70.8%)</td>
<td>0.36</td>
</tr>
<tr>
<td>MBL level Day 0*</td>
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<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>&lt;1000 µg/L</td>
<td>15 (37.5%)</td>
<td>6 (37.5%)</td>
<td>9 (37.5%)</td>
<td>0.57</td>
</tr>
<tr>
<td>&gt;1000 µg/L</td>
<td>24 (60.0%)</td>
<td>9 (56.4%)</td>
<td>15 (62.5%)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* 1 MBL serum level day 0 missing, 'IQR=interquartile range

### Results

**Patient characteristics of the total group:**

In total, 40 patients were entered in the study. The median age was 8.6 years (IQR 4.3-13.5 years). There were 27 males (67.5%) and 13 females (32.5%). Hematological malignancies were seen in 17 patients (42.5%) (16 patients ALL, 1 patient ANLL) and 23 patients (57.5%) had solid malignancies, of which rhabdomyosarcoma, neuroblastoma and Ewing sarcoma were the most frequent. A total of 9 patients were relapsed patients (table 2).
Mannan-Binding Lectin (MBL) serum levels in pediatric oncology patients

**MBL gene and serum level:**
Of these 40 patients, 34 gene sequences were performed. Twenty one patients (52.5%) were found to have a normal MBL gene, and 13 patients (32.5%) showed exon 1 mutations (Table 2). In 39 patients the MBL-level was done together with the geno typing at a time the patient was not febrile or neutropenic. In 13 patients (32.5%) levels <800 µg/L were found and 26 patients (65%) had levels >800 µg/L. With the use of a ROC curve on our own data the cut-off point was 1000 µg/L. There were 15 patients (37.5%) with a level <1000 µg/L, and 24 patients (60%) with a level >1000 µg/L. The median level of MBL in patients with no gene mutation was 3010 µg/L (IQR 1900-4385 µg/L). In patients with an MBL gene mutation in exon 1 the median level of MBL is 340 µg/L (IQR 90-715 µg/L). As expected, the difference of the MBL serum level between the patients with wild type MBL and the group with exon-1 mutations was significantly different (Mann-Whitney U test p=0.001)(Fig. 1).

![Figure 1: Scatter plot representing MBL-serum level in wild-type MBL group and exon-1 mutation group (n=40). All values are plotted, mean is illustrated, p=0.001.](image)

**No febrile neutropenic episode:**
During this study-period there were 16 patients (40%) who became neutropenic during the study-period but did not need to be admitted with a febrile neutropenic episode. In this group, there were 13 males (81.3%) and 3 females (18.8%). Six patients (37.5%) had hematological malignancies (ALL), 10 patients (62.5%) had solid malignancies. Eight patients (50.0%) had a wild-type MBL, 5 patients had exon 1 mutations (31.2%), 3 gene mutations were missing. Six patients (40%) had MBL levels <800 µg/L, and 9 patients (60%) had levels >800 µg/L. Using our own cut-off level the number of patients in each group did not change. There were no significant differences between this group and the group who did experience a febrile neutropenic episode (see Table 2).
Febrile neutropenic episode:

Patient characteristics:

Of the 40 patients, 24 were followed prospectively upon admission of the episode of febrile neutropenia (60%). The median age of this group of patients was 5.7 years (IQR 3.9-12.7 years). There were 14 males (58.3%) and 10 females (41.7%). Hematological malignancies were seen in 10 patients (45.8%) (9 patients had ALL, 1 patient ANLL) and 13 patients (54.2%) had solid malignancies (Table 2). Of the patients treated for a febrile neutropenic episode there were 6 relapse patients.

MBL gene and serum level:

Of the 24 patients 21 were genotyped. Of these patients 13 had a normal MBL gene (54.2%) and 8 patients had exon-1mutations (33.3%) (Table 2).

Seven patients (29.2%) had MBL levels <800 µg/L, and 17 patients (70.8%) had MBL levels >800 µg/L, measured before the febrile neutropenic episode. Using the cut-off measured with the ROC, 9 patients (37.5%) had MBL-levels <1000 µg/L and 15 patients (62.5%) had MBL-levels >1000 µg/L. Of this group of patients MBL levels were measured prospectively on day 1, 3 and 5 of the febrile neutropenic episode during hospitalization. The MBL levels of MBL-deficient children did not rise during this time period. On day 1 the median MBL level was 370 µg/L (IQR 145-850 µg/L), 485 µg/L (IQR 100-1470 µg/L) on day 3, and 635 µg/L (IQR 120-2317µg/L) on day 5, respectively (t-test for paired samples N.S.). In the MBL-sufficient children there was a slight yet nonsignificant rise in MBL-levels measured between day 1, 3 and 5. On day 1 the median MBL level was 2300 µg/L (IQR 1750-3910 µg/L), 3060 µg/L (IQR 2000-5700 µg/L) on day 3 and 2500 µg/L (IQR 2170-4150 µg/L) on day 5, respectively (N.S.)(Figure 2).

Severity of the febrile neutropenic episode:

Of the 24 patients 16 patients (66.6%) had a neutrophil count <100 cells/mm$^3$ (defined as severe neutropenia) on the first day of the febrile neutropenic episode. The median WBC on day 1 was 0.6 x 10$^9$/L (IQR 0.2-1.1 x10$^9$/L). All patients were started on selective gut decontamination before the onset of neutropenia. At the onset of fever during neutropenia all patients were admitted and started on broad spectrum intravenous antibiotics (either vancomycin or a second generation cephalosporin combined with gentamicin). Concerning the outcome of the neutropenic episode, 5 patients (20.8%) had a positive blood culture (4 Gram-positive organisms and 1 Gram-negative organism), 4 patients (16.6%) were admitted to the intensive care unit, 2 patients (8.3%) died (one patient died because of infectious complications, the other patient developed a severe cardiomyopathy during the induction phase of ANLL treatment).

Wild-type MBL patients compared with patients with exon-1 mutation:

The number of patients with a neutrophil count <100 cells/mm$^3$ was not significantly different
between the 2 groups, however, the duration of neutropenia showed a trend towards significance (p=0.07). Even in this small cohort of patients (n=24) there are more patients who experience a longer duration of neutropenia in the exon-1 mutation group (Table 3).

Clinical characteristics (respiratory rate, lung problems, gastrointestinal problems) at the onset of the febrile neutropenic episode were not significantly different between the 2 groups (not shown). The fever pattern (peaking, continuous or rapidly normalising) and the duration of fever (quick recovery (1-3 days), moderate (3-7 days), severe (>7 days)) were not significantly different between both groups (Table 3).

Also the number of positive blood cultures, ICU admissions and death of the patient were not significantly different between both groups (Table 3).

If all analyses were done comparing the MBL-deficient group (MBL<1000 µg/L) to the patients with an MBL level >1000 µg/L, no significant differences were found. There was however a trend that more positive blood cultures were found in the MBL-insufficient group (37.5%) compared to 13.3% in the MBL-sufficient group (chi square, N.S. p=0.29). The same trend was observed comparing the duration of fever in the 2 groups. In the MBL-insufficient group 50% of patients had a duration of fever >3 days, in the MBL-sufficient group this concerned 26.6% (chi square, N.S. p=0.25).

**Laboratory parameters in patients related to the MBL genotype:**

The Hb level, platelets, leucocytes, and liver enzymes were not significantly different between the MBL-deficient and the MBL-sufficient group (not shown).

However, the CRP values on day 1 of the FNE between both groups was significantly different (t-test p=0.015). It was shown that patients with an exon-1 mutation had a significantly lower
Table 3: Comparison of the MBL wild type group and exon-1 mutation group in the febrile neutropenic episode group (n=24)

<table>
<thead>
<tr>
<th></th>
<th>MBL wild type</th>
<th>MBL mutation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time from diagnosis to febrile neutropenic episode</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median 6.6 months</td>
<td>Median 12 months</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Range 0-23</td>
<td>Range 2-19</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Relapse patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (15.4%)</td>
<td>3 (42.8%)</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Neutro &lt;100 cells/µL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (69.3%)</td>
<td>5 (71.4%)</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>&gt;100 cells/µL</td>
<td>4 (30.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration neutropenia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 days</td>
<td>7 (53.8%)</td>
<td>3 (42.8%)</td>
<td>0.07</td>
</tr>
<tr>
<td>5-7 days</td>
<td>6 (46.2%)</td>
<td>4 (57.2%)</td>
<td></td>
</tr>
<tr>
<td>&gt;7 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fever pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaking</td>
<td>4 (30.7%)</td>
<td>1 (14.2%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Continuous</td>
<td>2 (15.3%)</td>
<td>1 (14.2%)</td>
<td></td>
</tr>
<tr>
<td>Rapidly normal</td>
<td>7 (54.0%)</td>
<td>5 (71.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of fever</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 days</td>
<td>9 (69.2%)</td>
<td>5 (71.4%)</td>
<td>0.55</td>
</tr>
<tr>
<td>3-7 days</td>
<td>2 (15.4%)</td>
<td>2 (28.6%)</td>
<td></td>
</tr>
<tr>
<td>&gt;7 days</td>
<td>2 (15.4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Blood-culture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-positive</td>
<td>2 (15.4%)</td>
<td>2 (28.5%)</td>
<td></td>
</tr>
<tr>
<td>Gram-negative</td>
<td>0</td>
<td>0</td>
<td>0.69</td>
</tr>
<tr>
<td>No growth</td>
<td>11 (84.6%)</td>
<td>5 (71.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;150 mg/L</td>
<td>4 (30.8%)</td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>&lt;150 mg/L</td>
<td>9 (69.2%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>ICU admission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (30.8%)</td>
<td>0</td>
<td>0.13</td>
</tr>
<tr>
<td>No</td>
<td>9 (69.2%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td>2 (15.4%)</td>
<td>0</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* 1 relapse patient genotype missing

CRP (median 50 mg/L IQR 12-86 mg/L) than patients with the wild type MBL genotype (median 93 mg/L IQR 60-210 mg/L Figure 3). This difference did not remain during the total febrile neutropenic episode.

Discussion

In this small prospective cohort study in pediatric oncology patients, the incidence of mutations of the MBL exon 1 gene was found to be 32.5% and the incidence of MBL-deficiency (<800 µg/L) was 32.5%. These findings are in agreement with earlier findings of prospective studies in cancer patients. Although the patients with an exon 1 mutation had low serum levels of
Mannan-Binding Lectin (MBL) serum levels in pediatric oncology patients

Figure 3: Scatter plot representing the differences in CRP level comparing patients with MBL <1000 ig/L (I) with patients who have an MBL levels >1000 ig/L (II). The mean is represented. The differences in levels between both groups are significant (p=0.015).

MBL, during febrile neutropenic episodes these patients did not have a more severe infections as was found before. There was a trend however to a prolonged duration of neutropenia as was found in the study of Neth et al. All our other findings on severity of infection were in keeping with Bergmann et al and Kilpatrick et al who found that there was no strong relationship between MBL and chemotherapy-related infections. Bergmann et al who studied 80 adult ANLL patients, hypothesized that these patients have such a severe and prolonged neutropenia that the effector function of MBL is severely compromised. The main effector functions of MBL enhance phagocytosis through complement receptors expressed on macrophages, monocytes and neutrophils and through complement activation this in turn will influence the intracellular fate and the inflammatory response. Because of the existing severe neutropenia, the possible lack of MBL may be completely overshadowed. This same result was shown in patients with a primary phagocytic disorder, such as chronic granulomatous disease. These patients also did not show a significant relation between MBL deficiency and severity of infections. The above could well be the explanation for this hemato-oncological cohort of children used. Most of the included patients were not "newly diagnosed" patients but had been on chemotherapy at least several months (median 8 months, range 0-59 months). The early induction phase is the worst period in which MBL-deficiency may be more relevant. Six patients in the febrile neutropenic episode group were relapsed patients who were now experiencing chemotherapy for the second time. Of all patients, 70% presented with a severe neutropenia (<100 cells/mm³). However, we are aware that we are dealing with a small cohort of patients in this study, in which the exon-1 mutations are known but the polymorphisms of the promoter region are not performed as yet. The results of the promoter polymorphisms might change the results, but we do not expect major changes in outcome because of the
strong linkage disequilibrium between the promoter region polymorphisms and exon-1 variants of the MBL2 gene. If we analyzed the results according to a cut-off level found in this cohort of patients (ROC curve level 1000 μg/L) there is a non-significant trend towards more positive blood-cultures in the deficient group (37.5%) compared to the sufficient group (13.3%), and longer duration of fever >3 days in the deficient group (50%) compared to the sufficient group (26.6%). Extending the cohort of patients might clarify the meaning of above results. Power analysis would predict significance if 150 patients were included in the total cohort. In our cohort of patients the MBL-deficient patients did not show a rise in their MBL serum levels during the febrile episode. The MBL-sufficient patients did show a slight but not significant increase in MBL serum level during the febrile episode. As MBL is known as a protein of the acute phase response one expected to see a rise in MBL levels over time. Consistent with this finding is the fact that the CRP values of the MBL-deficient patients were relatively low (<150 mg/L) at the start of the febrile episode. This cut-off point was chosen because in earlier studies this was shown to be a good predictor of bacteremia's in patients with cancer. In the MBL-deficient group no ICU admissions were observed and no deaths. This is probably the explanation why no high CRP values was found.

Of course it is known that both MBL and CRP act as acute phase proteins, and bind to specific ligands found on the surface of certain bacteria, and both may separately activate the complement system. MBL activates the complement cascade via the lectin pathway, and CRP activates complement via the classical route, and at the same time inhibits the activation of the alternative pathway. The complement activating functions of CRP and MBL may be coordinated in the acute-phase response. This could be one of the explanations to find low CRP values in the MBL-insufficient group, even though septicemia occurred relatively more often.

These preliminary data in a relatively small cohort of hemato-oncological children does not show significant impact of MBL genotype or serum levels on the chosen end-points. Yet, the data can not exclude that MBL suppletion, starting prior to the occurrence of bone-marrow depression may work by mechanisms and principles different from the end-points studied. Possible ways might still be, prevention or shorter duration of fever in the MBL-deficient patients, shorter duration of neutropenia, limitation of the toxic effects of chemotherapy on neutrophil development resulting in an earlier recovery, and reduction of toxic effects on gastro-intestinal leakage and permeability, thus enhancing barrier function of mucosa.

In sum, these effects may promote the well-being or recovery of the patient at a level different from infectious disease. Of course the success of MBL may be overshadowed by the increased intensity of the chemotherapy regimens during the last 5 years. A prospective trial on MBL substitution will necessitate strict inclusion criteria to determine the benefit of MBL substitution in this group of patients.
Reference List


Chapter

Pseudomembranous and neutropenic enterocolitis in pediatric oncology patients

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Abstract:

Neutropenic enterocolitis in oncological patients represents a wide spectrum of clinicopathological pictures each with its own entity. Early diagnosis of enterocolitis can lead to improved supportive care and therefore better outcome. We present 2 cases, case A - a child with pseudomembranous colitis caused by *Clostridium Difficile* and case B - a child with neutropenic enterocolitis, where no organism was found. By gaining insight in the pathology, immunology and culture results we demonstrate that early diagnosis leads to an improved management and therefore improved outcome.
Introduction

The nomenclature of enterocolitis in oncology patients remains difficult and encompasses the pseudomembranous colitis most often caused by *Clostridium difficile* infection and the neutropenic enterocolitis (also called ileo-cecal syndrome, agranulocytic colitis or typhlitis) most often caused by other causes. Clinical signs and symptoms vary widely from mild infection to severe transmural colitis with a high mortality-rate [12].

We report 2 cases demonstrating neutropenic enterocolitis in the pediatric oncology patient. Understanding of these enterocolitis entities in an earlier stage of the disease, concerning the pathological, inflammatory and culture results will enable us to support these patients more adequately and therefore decrease the morbidity and mortality due to this complication.

Case A

A 17 year old boy presented with an Ewing sarcoma of the 7th left rib. Treatment was started on the high-risk Ewing protocol (EICES 92 –European Intergroup Cooperative Ewing’s Sarcoma Study). Chemotherapy was given consisting of etoposide, vincristin, adriamycin, and ifosfamide. After the first course of chemotherapy, he became neutropenic with a WBC of 0.2 x 10^9/L. He developed fever (>39 °C) and was admitted. Treatment with i.v. vancomycin and gentamycin was started. Twenty-four hours after starting these antibiotics, he developed profuse diarrhea and complained of severe abdominal pain with cramps.

A rectoscopy was performed which showed mucosa covered with membranes, (see Figure 1). Microscopically the histopathological changes fitted the diagnosis of pseudomembranous colitis. Stool cultures were positive for *C. difficile* and *C. difficile* toxin (B). No viruses, parasites or other bacteria were isolated. His blood-cultures were negative. Serum parameters for inflammation were determined. On the first day, low values of elastase (47 ng/L), IL-6 (85 ng/L), and IL-8 (194 ng/L) were found.

He was started on oral metronidazol 3 times daily, and adequate supportive care was given. On the third day a second rectal biopsy was performed which still showed pseudomembranes. Clinically he improved. The abdominal pain was less, his diarrhea less profuse, and his WBC started to recover. After a total period of 8 days his intravenous antibiotics were stopped, the metronidazol was continued for 14 days.

On stopping the metronidazol a third rectal biopsy was performed and showed a complete recovery of the mucosa. The patient is now in complete remission and doing well, he does not have any abdominal complaints.
Case B

A 6 year old boy presented with an abdominal Burkitt lymphoma. He was started on the LMB 96 protocol (Lymphoma protocol) consisting of cyclofosfamide, vincristin, prednisolon, doxorubicin and methotrexate. Two weeks after the first course of chemotherapy he became severely neutropenic with a WBC of 0.1 x 10^9/L and no neutrophils. The Hb was 9.5 gr/dl, and the platelet count was 36 x 10^9/L. He developed fever and severe abdominal pain with a distended abdomen. He had bloody diarrhea and showed signs of sepsis with a low blood-pressure. Intravenous antibiotic therapy consisting of vancomycin and gentamycin was started. He was transferred to the intensive care unit for inotropic support. A rectal biopsy was performed. Macroscopically this showed severe mucosal lesions, and microscopically ulcerative changes with fibrinous exudate was shown with only a slight inflammatory reaction, representing a neutropenic enterocolitis (see Figure 2). Stool cultures were negative for bacteria, parasites and viruses. C. difficile toxin tests were negative, blood cultures were also negative. On the day of the biopsy, the CRP was elevated to 280 mg/L, other markers for inflammation showed a low elastase (62 ng/L), a moderately raised IL-6 (139 ng/L), and a high IL-8 (2391 ng/L). He continued with intravenous antibiotics and metronidazol was added orally even though no clostridium species were cultured. His oral feeds were stopped and he was started on parenteral nutrition. On day 5 a second rectal biopsy was performed and still showed mucosa covered with exudate, confirmed on histopathology. On day 5 the markers of inflammation showed a rise in elastase to 316 ng/l and a decrease in IL-6 (15 ng/L) and IL-8 (21 ng/L). The neutrophil count improved and he improved clinically, without surgical intervention. He completed his chemotherapy courses without subsequent episodes of enterocolitis. He is in complete remission and doing well.
Pseudomembranous colitis

Epidemiology

*Clostridium difficile* has become one of the most important hospital pathogens of the 90’s. Anand and Glatt [1] reviewed that antibiotics are not the only agents capable of inducing *C. difficile* associated diarrhea. They reported on 23 oncology cases. A variety of anti-neoplastic agents were involved, most commonly methotrexate. Chemo-therapeutic agents alter the gut flora and this is most likely the predisposing factor. Any compound that affects the gastro-intestinal flora, either qualitatively or quantitatively, may reduce the colonization resistance and therefore predispose the individuals to infection with *C. difficile*.

The normal carriage rate in adults is 0-3%. In oncology units the carriage rate increases to 13-28% in hospitalized patients. Schuller et al [11] looked at prevalence of *C. difficile* infection on a pediatric oncology unit over a period of one year. The carriage rate was 13% as was found in the literature. Of these children 68% had signs and symptoms attributable to *C. difficile* infection. The authors conclude that the organism is probably endogenous and is provided with a favorable environment by the combination of cancer chemotherapy and broad spectrum antibiotics [10].

Clinical findings

Clinical findings in *C. difficile* enterocolitis can range from absolutely asymptomatic to severe colitis. In view of the discussion we will restrict us to the severe colitis syndromes. Symptoms are profuse debilitating diarrhea, abdominal pain and distension. Common systemic manifestations include fever, nausea, anorexia, malaise and dehydration. In the most severe situation this form of colitis can present as an acute abdomen. The patients are extremely ill with lethargy, fever, tachycardia and abdominal pain. The colonic muscular tone may be lost resulting in toxic dilatation or megacolon, eventually leading to colonic perforation and peritonitis [8].
Pathology
A grading system for pseudomembranous colitis was proposed by Price and Davies [9]:

The Type-I lesion consists of focal necrosis of interglandular superficial intestinal epithelium with overlying neutrophilic exudates.

The Type-II lesion results from fusion of this neutrophilic exudate over the necrotic upper parts of the neighboring glands and in the late part of this phase neutrophilic exudates are nearly confluent over mucosa, (pseudomembranes).

The Type-III lesion shows complete coagulative necrosis of the intestinal mucosa.

When the C. difficile infection resolves the mucosa returns to normal.

Pathogenesis
The underlying pathogenesis involves a disruption of the normal bacterial flora of the colon, followed by colonization with C. difficile and the release of toxins causing mucosal damage and inflammation [4, 8].

Laboratory diagnosis
The golden standard at presentation is the stool cytotoxin test. It is a tissue culture assay based on the induction of cell rounding by C. difficile-toxin B in stool infiltrate. This assay has a high sensitivity (94-100%) and high specificity (99%) [4]. A stool culture for C. difficile is less efficient as many strains are non-toxicogenic. Rapid enzyme immunoassay’s have been developed detecting both toxin A and B (sensitivity 69-87% and specificity 99-100%) [4].

Therapy
Specific treatment aimed at eradicating C. difficile is used if symptoms are severe or persistent. Oral metronidazole and oral vancomycin are used as the drugs of choice. Both are equally effective. There is no difference in the rates of response, relapse or failure between these agents. Patients who cannot take oral medication can be treated with intravenous metronidazol. Excretion of the drug in the bile and exudation from the inflamed colon result in bactericidal levels in the faeces. Intravenous vancomycin is not indicated [4].

Neutropenic enterocolitis (Typhlitis)
Epidemiology
Typhlitis involves chemotherapy induced damage to the intestinal mucosa, primarily in the terminal ileum, ascending colon and cecum, and occurs when these patients are neutropenic [12].

In 1933 this entity was first described by Cooke when she described submucosal hemorrhage and appendiceal perforation in children with leukemia. Then from the 1960’s onwards autopsy studies reported an incidence of 12% to 46 % in leukemic children.
Clinical findings
Most patients are neutropenic more than one week before the onset of abdominal pain. Abdominal pain is diffuse in most cases and in a few cases localized to the right lower quadrant. The classical triad of symptoms is high fever, abdominal pain and diarrhea [13].

Organisms
The organisms involved are mainly Gram-negative organisms, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp, *Enterococci*, and Gram-positive organisms like the *C. difficile* and the *C. septicum*. Fungal organisms can also be cause of severe neutropenic enterocolitis. In many cases no organism can be isolated [6].

Pathophysiology
This remains largely unknown but is believed to be multi-factorial:
- Destruction of the normal mucosal architecture due to chemotherapy and/or radiotherapy with possible coexistent leukemic or lymphomatous infiltrates.
- Intramural hemorrhage due to severe thrombocytopenia.
- A shift in the normal gastrointestinal microbial flora due to antibiotics, antifungals and nosocomial colonization by hospital flora [6].

Pathology
The bowel appears thickened and edematous, with scattered ecchymoses on the serosal surface and ulceration on the mucosal surface. Microscopically there is hemorrhage necrosis involving the mucosa and submucosa with striking scarcity of acute inflammatory reaction, few or no granulocytes. There may be an infiltration of bacteria or fungi. Sometimes an exudate resembling pseudo-membranes consisting of fibrin and cell debris may be found overlying the most severely ulcerated mucosal surfaces.
In later stages, the process may progress to involve the full thickness of the bowel wall and sometimes lead to perforation [14].

Laboratory findings
Routine laboratory tests are of little value in diagnosing typhlitis. Blood and stool cultures are important to identify the organisms involved in the process of enterocolitis.

Therapy
The main mode of therapy is supportive care. Nasogastric suctioning, broad-spectrum antibiotics, administration of appropriate blood products and adequate fluid replacement.
4 criteria have been used for surgical intervention:
Persistent gastrointestinal bleeding and thrombocytopenia and clotting abnormalities. Evidence of free intraperitoneal perforation.

Clinical deterioration requiring support with vasopressors or large volumes of fluid suggesting uncontrolled sepsis.

Development of symptoms of an intra-abdominal process in the absence of neutropenia which would normally require surgery.

The presence of localized peritoneal signs is not an adequate indication for exploration. With these criteria in mind most patients can be treated without surgery. The most important in these patients is the requirement of neutrophils. Growth factors are often indicated [12]. Differences between pseudomembranous colitis and typhlitis are summarized in Table 1.

**Table 1: To compare typhlitis and pseudomembranous colitis:**

<table>
<thead>
<tr>
<th>Location</th>
<th>Typhlitis</th>
<th>Pseudomembranous colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical findings</td>
<td>Cecum, terminal ileum, proximal colon</td>
<td>Colon, rectum</td>
</tr>
<tr>
<td>Pathology</td>
<td>Abdominal pain, diarrhea</td>
<td>Abdominal pain, diarrhea</td>
</tr>
<tr>
<td></td>
<td>Often instable vital signs</td>
<td>Mostly stable vital signs</td>
</tr>
<tr>
<td>Pathology</td>
<td>Hemorrhage necrosis of mucosa</td>
<td>Pseudomembranes covering the intestinal mucosa</td>
</tr>
<tr>
<td></td>
<td>and submucosa</td>
<td></td>
</tr>
<tr>
<td>Organisms</td>
<td>Gram-negative organisms, fungal organisms</td>
<td>Clostridium difficile</td>
</tr>
<tr>
<td>Outcome</td>
<td>High mortality rate</td>
<td>Low mortality, high morbidity</td>
</tr>
<tr>
<td>Therapy</td>
<td>Supportive care and antibiotics covering the organism found</td>
<td>Supportive care and oral metronidazol or oral vancomycin</td>
</tr>
</tbody>
</table>

**Discussion**

With the increasing intensification of chemotherapy, toxicity during neutropenic episodes can be expected. Therefore familiarity with the spectrum of diseases encompassing neutropenic enterocolitis is needed to recognize the disease entities. Recognizing clinical signs, pathological changes and inflammatory changes might facilitate early detection, improve the supportive care around this spectrum of diseases and ultimately decrease the morbidity and mortality.

The 2 cases discussed both illustrated damage to the intestinal mucosa. In both cases rectoscopy was done as part of a prospective study gaining insight in the pathological, infectious and immunological causes of this complication. It was performed by the pediatric gastroenterologist with a flexible pediatric scope. Platelets were kept >50 x 10⁹/L and no complications were seen. Both cases were children with solid tumors. Neutropenic enterocolitis used to be an infrequent finding in patients with solid tumors [6].

Now with the intensification of chemotherapy this is no longer the case. It has been proposed that some chemotherapeutic agents cause direct epithelial necrosis in the gastrointestinal tract [5]. Following the damage to the mucosa secondary infections can occur due to enteric or opportunistic organisms like *pseudomonas* or fungal species [3]. The first patient described
developed a *C. difficile* colitis, possibly due to a shift in the normal gastrointestinal microbial flora due to antibiotics, antifungals and nosocomial colonization with hospital flora. [6]. The toxins of *C. difficile* cause direct toxic damage of the actinoskeleton leading to cell rounding, and eventually cell death [8].

The second patient described had ulcerative changes on rectal biopsy with a fibrinous exudate representing neutropenic enterocolitis. No organism was cultured. Both cases were managed conservatively and it was clearly shown that when neutrophils appeared in the blood the clinical picture improved [6].

Neutropenic enterocolitis should be suspected in a neutropenic patient with abdominal pain, fever and diarrhea. To define a definite enterocolitis rectoscopy was helpful in the 2 patients described. However mostly the typhlitis occurs in the cecum or sigmoid area which will not be adequately detected by rectoscopy. In this case the inflammatory parameters might be of use. Interleukin-8 was extremely high in the second patient illustrating the severity of the clinical condition. Interleukin 8 is an inflammatory chemokine which mainly functions as a neutrophil chemoattractant and activating factor [2] IL-6 and IL-8 have been used in neutropenic patients as a predictor of bacteremia in patients with fever during their neutropenic episode [2]. In patients with severe neutropenic enterocolitis, IL-8 release depends on toxic or ischemic bowel injury. The source therefore of IL-8 lies in the endothelial cells and fibroblasts, and not in the myeloid cells [7]. The high level of IL-8 together with the severe ulcerative changes on rectal biopsy in patient B acted as predictor of severity of the enterocolitis. Human neutrophil elastase is low when no neutrophils are present in the peripheral blood. A rise was observed on day 5 of the enterocolitis, soon after neutrophils were detected in the peripheral blood and the clinical picture of the patient improved. It was shown that neutrophil elastase increased just before the neutrophils recovered. Therefore elastase could act as predictor of clinical improvement (manuscript in preparation).

The clinical picture combined with the rectoscopy and the inflammatory parameters improves our understanding of this severe condition and enables us to support the patient in a more appropriate way, at an earlier stage of the disease.

**Acknowledgements**

We are grateful to our pediatric gastroenterology department for performing the rectoscopies and mucosal biopsies and to our pathology department for doing the microscopy.
References

2. de Bont ES, Vellenga E, Swaanenburg JC, Fidler V, Visser-van Brummen PJ, Kamps WA (1999) Plasma IL-8 and IL-6 levels can be used to define a group with low risk of septicaemia among cancer patients with fever and neutropenia. Br J Haematol 107:375-380
Chapter

Severity of enterocolitis predicted by IL-8 in pediatric oncology patients

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Abstract

Enterocolitis in oncology patients remains an important complication with poor insight in microbial, pathological and inflammatory aspects.

Pediatric oncology patients admitted with neutropenic fever, who developed abdominal pain and diarrhea, were monitored with rectal biopsies, cultures, and inflammatory markers.

Twenty-five patients were included (mean age 7.1 years). Eight patients (32%) needed intensive care treatment, 3 (12%) patients died. Gram-positive bacteraemia was diagnosed in 4 patients (16%). Most patients had negative blood and stool cultures. Predictors of a severe clinical course of the enterocolitis were an increased serum IL-8 (>1000 pg/mL) and an increased serum CRP (>150 mg/L), both measured on the first day of clinical illness. Relative risks for admission to ICU were 11.3 (95% CI 1.6 to 77.9) for elevated IL-8 and 6.4 (95% CI 0.92 to 45.1) for increased CRP. Rectal biopsies and pathology could not predict outcome (p=0.22).

IL-8 analysis at the onset of enterocolitis symptoms can identify high-risk patients, which might be used clinically to design future intervention trials.

1. Introduction

Neutropenic enterocolitis represents a complex spectrum of inflammatory processes of the colon seen in immunocompromised hosts after intensive chemotherapy for malignancies. It ranges from pseudo-membranous colitis caused by Clostridium difficile to typhilitis [1-3]. The clinical picture ranges from mild infection to severe transmural colitis with a high mortality-rate (50-100%) [4]. Neutropenic enterocolitis was initially defined as a clinical-pathological entity through retrospective review of autopsy findings in leukaemic patients first described by Cooke in 1933 [5]. The main causative organisms are Gram-negative bacilli, mainly Pseudomonas aeruginosa and Escherichia coli, followed by Clostridium difficile, Clostridium septicum and fungal pathogens [6]. The pathogenesis of this disorder is thought to be due to a multifactorial disruption of the mucosal barrier, in which the bacterial flora, neutropenia and cytotoxic therapy play a role. The invasive infection leads to ischaemia followed by necrosis of the various layers of the bowel wall. Although the process may have a pre-dilection for the terminal ileum and caecum any segment of the bowel can be involved [7,8].

Prognostic inflammatory markers in neutropenic enterocolitis have not been defined to date. Many studies have however evaluated prognostic inflammatory markers in patients with fever in neutropenia irrespective of any symptoms of an impending enterocolitis [9-11]. Low serum IL-8 levels at the onset of fever can define a low-risk subgroup of neutropenic patients who can safely be treated with antibiotic monotherapy instead of combination therapy [12]. In similar terms, Bont et al could define a low-risk group at the start of fever by the use of IL-8 and IL-6 as plasma parameters: the IL-8 and IL-6 plasma concentration were significantly increased in patients.
with chemotherapy-related neutropenia and fever due to bacteremia (mainly Gram-negative bacteremia) compared to fever of non-bacterial origin [10,13].

In neutropenic enterocolitis both toxic and ischemic bowel injury may play an important role. The response of the inflammatory cascade to pathogens attacking the gastro-intestinal mucosa involves mainly the cytokines IL-6, IL-8, IL-10 and TNF-α [14]. The role of these cytokines may be important in the pathophysiology of the inflammatory responses in neutropenic enterocolitis in children [11,15]. Therefore a prospective single unit study was started to identify the incidence of enterocolitis in a pediatric oncology unit, to gain insight in the pathogenetic mechanisms, and to identify clinical and inflammatory prognostic markers.

2. Methods

2.1 Patient selection

Entry criteria were neutropenic pediatric oncology patients who had abdominal pain, diarrhea and fever for more than 24 hours. Neutropenia was defined as <500 cells/mm³ absolute neutrophils. Diarrhea was defined as having at least grade 2 diarrhea (4-6 times in 24 hours correlating to the CTC toxicity criteria). Fever was defined as a temperature >38.5°C. All types of malignancies were included.

The study was performed in a single pediatric oncology unit. Approval from the medical ethics committee was obtained. Informed consent was obtained from the parents and from the child if >12 years of age.

2.2 Patient analyses

On the first day of the study: 1) history and clinical checklist 2) physical examination 3) stool cultures 4) rectal biopsy 5) blood investigations and 6) serum levels of inflammatory markers.

On day 3 and day 7 above investigations were repeated. The rectal biopsy was repeated if findings on day 1 were abnormal. The next course of chemotherapy started when all abnormal findings had normalized.

Ad 1). History and clinical checklist. The food intake of the week before onset of symptoms was recorded as well as medication before onset of symptoms. Clinical signs and symptoms of the illness (abdominal pain, nausea, diarrhea, blood loss and pattern of fever) were recorded.

Definitions used: Fever normalized within 48 hours was classified as “short duration”, fever which continued >48 hours was classified as “long duration” and if fever spiked above 38.5°C every 24 hours, and the temperature normalized in between this was classified as “spiking”. Abdominal pain was classified as “cramps”, “stool related”, or “continuous”, and the frequency of vomiting was recorded. Stools were recorded as “bloody stools” or as “watery frequent”.

Ad 2). Physical examination. State of consciousness, respiratory rate, hypotension, abdominal
distension, bowel sounds, mucositis, any other clinically important abnormalities were reported. Mucositis was recorded as present when there were oral lesions in the mouth. The clinicians scoring the history and physical examination were blinded for the inflammatory marker results and the results of the biopsy findings.

**Ad 3). Stool cultures.** Stool cultures were performed for bacteria, viruses, parasites, and fungi, using routine microbiological procedures. Faeces was tested for Clostridial cytotoxin. Detection of *C. difficile* was performed as follows. Faeces was tested by enzyme immuno-assay (Premier™ Toxins A&B, Meridian Bioscience, Ohio) to detect *C. difficile* toxins A and B. For culture faeces was pretreated by mixing with ethanol 96% during one hour. *C. difficile*-like colonies were identified using standard microbiological procedures.

**Ad 4). Rectal biopsy.** Rectal mucosal biopsies were taken by an experienced pediatric gastroenterologist. This was performed by a flexible adult sigmoidoscopy which was introduced no more than 15 cm, no anesthetics were needed; in smaller children conscious sedation was given 30 minutes before the procedure. The procedure was only done when the platelets were above 50 x 10⁹/L. If the platelets were <50 x 10⁹/L then a platelet-transfusion was given 30 minutes prior to the procedure. Insight was gained in the macroscopic and the microscopic aspects of the rectal mucosa. Microscopically four categories were distinguished in order of increasing severity: "no changes", "infiltrate only", "pseudomembranes" and "ulcerative changes with fibrin exudate". The diagnosis of *C. difficile*-related disease was made if 2 out of 3 of the following findings were included: pseudomembranes on biopsy, toxin-positivity in the stools and/or a positive culture of *C. difficile* either in the blood or stools.

**Ad 5). Blood investigations.** Full blood count, CRP, liver-enzymes, creatinin, viral serology and blood-cultures were performed, according to standard laboratory procedures.

**Ad 6). Inflammatory markers.** C-reactive protein (CRP) was measured in blood. This assay was performed as described by Wolbink et al. [16] using anti-CRP mAb KH61 (2 mg/ml) as the coating mAb, and biotinylated anti-CRP mAb 5G4 to detect bound CRP. IL-6, IL-8, IL-10 and TNFα concentrations were analyzed using commercially available ELISA’s with detection limits of 3.0 pg/mL, 15.0 pg/mL, 15 pg/mL, and 5.0 pg/mL, respectively (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands).

### 3. Statistics

To compare the Intensive Care Unit (ICU) group versus the non-ICU group the Mann Whitney U test was used on the continuous data and the chi-square test for categorical data; if the expected number of patients was below five in one of the cells we performed a Fisher exact test.

To examine the prognostic value of elevated inflammatory markers we used both cut-offs mentioned in the literature (for IL-8 above 1000 pg/mL [10], CRP above 150 mg/L [11]) and optimal cut-offs from the data were calculated using the Youden index (sensitivity + specificity -1) as criterion. To estimate the predictive value of increased inflammatory parameters we calculated relative risks
with 95% confidence intervals (CI). All reported p-values are two-sided.

4. Results

4.1 Patient characteristics

Over a 3-year period (November 1998 until January 2002), 452 new patients with oncological disorders were admitted to the unit. Of these 25 patients fulfilled the entry criteria of the study and were included, 15 were male and 10 were female. The diagnoses at presentation showed 11 hematological disorders (ALL/ANLL), 4 B-cell lymphoma’s, 9 solid tumors and 1 hemophagocytic syndrome.

The mean age at diagnosis was 7.1 years (range 1.0-17.1 years). Enterocolitis presented in most cases within the first 3 months after diagnosis and the start of chemotherapy.

The clinical symptoms are summarized in Table 1. The vast majority of patients had signs of mucositis (92%), 40% of patients had less than 48 hours of fever and 60% had fever >48 hours.

The stool pattern was recorded as “watery and frequent” in 60% of patients and in 28% of patients “bloody diarrhoea” was recorded. The abdominal pain was classified as “cramps” in 72% of patients, and 24% of patients had continuous pain.

Of the 25 patients, 8 were admitted to the ICU due to cardiovascular symptoms for which inotropic support was indicated. Of these patients five did not have a proven bacteremia. Three died following the enterocolitis episode, even though these patients received adequate antibiotic coverage and extensive inotropic support. The first patient with a hemophagocytic syndrome had pseudomembranous

<table>
<thead>
<tr>
<th>Table 1: Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Sex: Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Diagnosis:</td>
</tr>
<tr>
<td>Haematological</td>
</tr>
<tr>
<td>Lymphoma</td>
</tr>
<tr>
<td>Solid</td>
</tr>
<tr>
<td>Haemophagocytic syndrome</td>
</tr>
<tr>
<td>CVC:</td>
</tr>
<tr>
<td>PAC</td>
</tr>
<tr>
<td>Broviac</td>
</tr>
<tr>
<td>No CVC</td>
</tr>
<tr>
<td>Clinical parameters:</td>
</tr>
<tr>
<td>Fever:</td>
</tr>
<tr>
<td>&lt;48 hours</td>
</tr>
<tr>
<td>Long and spiking</td>
</tr>
<tr>
<td>Long and continuous</td>
</tr>
<tr>
<td>Diarrhea:</td>
</tr>
<tr>
<td>Watery and frequent</td>
</tr>
<tr>
<td>Bloody</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Abdominal pain:</td>
</tr>
<tr>
<td>Cramps</td>
</tr>
<tr>
<td>Stool related</td>
</tr>
<tr>
<td>Continuous pain</td>
</tr>
<tr>
<td>Mucositis:</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Vomiting:</td>
</tr>
<tr>
<td>&lt;4x/24 hours</td>
</tr>
<tr>
<td>&gt;4x/24 hours</td>
</tr>
<tr>
<td>no vomiting</td>
</tr>
<tr>
<td>Pathology results:</td>
</tr>
<tr>
<td>No changes</td>
</tr>
<tr>
<td>Infiltrate only</td>
</tr>
<tr>
<td>Pseudomembranes</td>
</tr>
<tr>
<td>Ulcerative changes</td>
</tr>
<tr>
<td>Outcome:</td>
</tr>
<tr>
<td>ICU admission</td>
</tr>
<tr>
<td>Mortality</td>
</tr>
</tbody>
</table>

CVC= central venous catheter, PAC = port-a-cath internal CVC device, Broviac= external CVC device. Pathology results are ranked to severity. In the outcome the Intensive Care Unit (=ICU) admission is described, and mortality due to enterocolitis.
colitis due to *C. difficile*, she underwent surgical resection of an ischemic part of the bowel but her condition deteriorated and she died. The 2 other patients suffered from acute myeloid leukemia, and had positive blood cultures at the time of colitis (*Candida tropicalis* and *Staphylococcus aureus*). They both deteriorated rapidly and died, despite appropriate antibiotic coverage and inotropic support. In all patients cardiomyopathy was excluded by echocardiography.

**4.2 Etiology**

Microbiological results are shown in Table 2. A variety of organisms were cultured but most stool and blood cultures remained negative (76% and 68%, respectively). Four patients had a Gram-positive bacteremia, none of these patients had a positive stool culture. One patient had an adenovirus in the feces, the blood culture remained negative, 2 patients had protozoa in the feces, one of these patients also had a Gram-negative bacteremia (*Klebsiella spp*). Only 2 patients had *C. difficile* in the stool culture and 1 of these patients had a positive blood culture with *C. difficile* (the same strain was found in the blood-culture as in the stool-culture). In 3 patients *C. difficile* toxin stool tests were positive.

Histological examination of the rectal biopsies resulted in "no changes" in 12 patients (48%), "infiltrate only" without pseudomembranes in 9 patients (36%), "pseudomembranes" in 3 patients (12%) and 1 patient had "ulcerative changes" with fibrin exudates (4%). Overall in 4 patients (16%) two out of three parameters for *C. difficile*-positive colitis were found to be positive.

<table>
<thead>
<tr>
<th>Table 2: Microbiological results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stool culture</strong></td>
</tr>
<tr>
<td>- Bacterial</td>
</tr>
<tr>
<td>- Gram-pos</td>
</tr>
<tr>
<td>- Gram-neg</td>
</tr>
<tr>
<td>Parasitic</td>
</tr>
<tr>
<td>Viral</td>
</tr>
<tr>
<td>Negative</td>
</tr>
</tbody>
</table>

* Blood-culture

- Bacterial
  - Gram-pos
    - *Strep. sanguis* | 2
    - *Staph. aureus*  | 1
    - *Coagulase-neg. Staphylococcus* | 1
    - *Clostridium difficile* | 1
  - Gram-neg
    - *Klebsiella spp.* | 1
    - *Escherichia coli* | 1
- Fungal
  - *Candida tropicalis* | 1
- Negative
  - 17

* Stool and blood culture results obtained during the same study period.
To explain the low rate of \textit{C. difficile} positive-colitis we analyzed the use of intravenous antibiotics prior to study inclusion. 19 patients (76\%) had received i.v. antibiotics prior to the rectal biopsy (mean of 5.5 days), these antibiotics were administered because of fever in neutropenia of which 13 patients (52\%) had been pretreated with vancomycin (Fig.1). Whereas none of these patients pretreated with vancomycin were \textit{C. difficile}-positive, 5 patients of this group showed abnormal rectal biopsies. The pathological changes, the negative stool-cultures and the relative small number of positive blood-cultures suggest a multifactorial etiology of neutropenic enterocolitis.

![Diagram](image_url)

\textbf{Fig. 1:} Intravenous use of antibiotics at the start of the study and biopsy results. The chart shows the number of patients pretreated with antibiotics, the rectal biopsy and \textit{Clostridium} test results.

**4.3 Prognostic factors**

To identify possible factors predictive of the clinical course we used admittance to ICU with need for inotropic support during the enterocolitis episode as our primary endpoint.

**4.3.1 Clinical parameters**

Patients included in the ICU group were 3 patients with ANLL, 3 patients with Burkitt lymphoma, one patient with high risk leukemia, and one patient with a hemophagocytic syndrome. In the non-ICU group there were 3 patients with ANLL, 1 Burkitt lymphoma, 4 patients with leukemia and 9 solid tumors. There was no difference in chemotherapy used between the two groups. The chemotherapy used in both groups included high dose cytosar, daunorubicin, etoposide,
Table 3: Clinical risk factors for admittance to ICU

<table>
<thead>
<tr>
<th></th>
<th>ICU</th>
<th>Non-ICU</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (25)</td>
<td>8 (32%)</td>
<td>17 (68%)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Abdominal pain:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cramps</td>
<td>4 (50%)</td>
<td>14 (82%)</td>
<td>p=0.15</td>
</tr>
<tr>
<td>Stool-related</td>
<td>1 (12.5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>3 (37.5%)</td>
<td>3 (18%)</td>
<td></td>
</tr>
<tr>
<td>- Diarrhea*:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watery frequent</td>
<td>4 (50%)</td>
<td>12 (70%)</td>
<td>p=0.34</td>
</tr>
<tr>
<td>Bloody</td>
<td>4 (50%)</td>
<td>3 (18%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0 (0%)</td>
<td>2 (12%)</td>
<td></td>
</tr>
<tr>
<td>- Fever:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short and continuous</td>
<td>0 (0%)</td>
<td>10 (59%)</td>
<td></td>
</tr>
<tr>
<td>Long and spiking</td>
<td>3 (37.5%)</td>
<td>3 (18%)</td>
<td></td>
</tr>
<tr>
<td>Long and continuous</td>
<td>5 (62.5%)</td>
<td>4 (23%)</td>
<td>p=0.15</td>
</tr>
<tr>
<td>- Mucositis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (100%)</td>
<td>15 (88%)</td>
<td>p=0.34</td>
</tr>
<tr>
<td>No</td>
<td>0 (0%)</td>
<td>2 (12%)</td>
<td></td>
</tr>
<tr>
<td>Blood culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-positive</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Gram-negative</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI toxin positive</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rectal biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Infiltrate only</td>
<td>2 (25%)</td>
<td>7 (41%)</td>
<td></td>
</tr>
<tr>
<td>- Pseudomembranes</td>
<td>2 (25%)</td>
<td>1 (6%)</td>
<td>p=0.22</td>
</tr>
<tr>
<td>- Ulcerative changes</td>
<td>1 (13%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- No changes</td>
<td>3 (37%)</td>
<td>9 (53%)</td>
<td></td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- WBC</td>
<td>0.25 x 10^9/L (0.1-0.52)</td>
<td>0.30 x 10^9/L (0.2-0.5)</td>
<td>p=0.39</td>
</tr>
<tr>
<td>- Platelet count</td>
<td>39 x 10^9/L (22-48)</td>
<td>32 x 10^9/L (14-83)</td>
<td>p=0.79</td>
</tr>
<tr>
<td>- SGOT</td>
<td>45 U/L (6.44)</td>
<td>17 U/L (11-25)</td>
<td>p=0.59</td>
</tr>
<tr>
<td>- creat</td>
<td>26 mg/L (16-35)</td>
<td>24 mg/L (18-37)</td>
<td>p=0.97</td>
</tr>
<tr>
<td>inflammatory parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CRP (day 1)</td>
<td>239 mg/L (239-292)</td>
<td>118 mg/L (85-188)</td>
<td></td>
</tr>
<tr>
<td>(day 3)</td>
<td>160 mg/L (82-212)</td>
<td>75 mg/L (27-146)</td>
<td></td>
</tr>
<tr>
<td>(day 7)</td>
<td>85 mg/L (10-219)</td>
<td>24 mg/L (6.3-72)</td>
<td></td>
</tr>
<tr>
<td>-IL-8 (day 1)</td>
<td>2882 pg/mL (1228-4536)</td>
<td>146 pg/mL (66.7-213.5)</td>
<td></td>
</tr>
<tr>
<td>(day 3)</td>
<td>393 pg/mL (157-1708)</td>
<td>41 pg/mL (30.5-113)</td>
<td></td>
</tr>
<tr>
<td>(day 7)</td>
<td>221 pg/mL (11.6-430)</td>
<td>31 pg/mL (15-33)</td>
<td></td>
</tr>
</tbody>
</table>

Prednisone and methotrexate. Abdominal pain, stool pattern, amount of vomiting and mucositis between the ICU and the non-ICU group were compared and no significant difference between the two groups was found. The pattern of fever however did show a difference, if fever was grouped in 2 categories, (fever <48 hours after onset of the enterocolitis, and fever>48 hours after onset colitis) In the ICU group 5 out of 8 patients (63%) had fever >48 hours compared to 4 out of 17 patients (23%) in the non-ICU group, this was borderline significant (p=0.056). In the original classification, in 3 categories no significant difference was found between the 2 groups (Table 3).
4.3.2 Microbiological results, rectal biopsy and other laboratory results

Both stool and blood cultures did not show a significant different pattern in the ICU group compared to the non-ICU group. The white cell count and platelet count, liver functions and renal function were also not significantly different in both groups. The histopathological results in both groups were not significantly different either (Table 3).

4.3.3 Inflammatory markers

Of the evaluated inflammatory serum markers CRP and IL-8 showed a significant difference between the 2 groups (Fig. 2 and 3). The median CRP level in the ICU and the non-ICU group was 239 mg/L (Interquartile range IQR 193 to 292 mg/L) versus 88.2 mg/L (IQR 119-187 mg/L), respectively (p=0.023). There was however overlap between the two groups (Fig. 2). At a cut-off level of 150 mg/L ([11], ROC cut-off 190 mg/ml), 7 out of 8 ICU patients had a high CRP compared to 6 out of 17 patients in the non-ICU group. Patients with a CRP > 150 mg/L had a 6.4 times higher chance of developing a severe enterocolitis compared to patients with a CRP<150 mg/L, (95%CI 0.92-45.1). Using the ROC cut-off level of this cohort of patients (190 mg/L) the chance of ICU admission was 10.5x higher (95% CI 1.5-72.8) compared to patients with a CRP<190mg/L.
### Table 4: IL-8 as prognostic tool in neutropenic enterocolitis

<table>
<thead>
<tr>
<th></th>
<th>IL-8&gt;1000 pg/mL</th>
<th>IL-8 &lt;1000 pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU</td>
<td>6 *</td>
<td>1</td>
</tr>
<tr>
<td>Non-ICU</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>15</td>
</tr>
</tbody>
</table>

* All 3 patients who died were found in this group. Relative Risk 11.3 (95% CI 1.6-77.9) (There are 2 missing values,). If the ICU missing IL-8 value was <1000 pg/mL and the missing IL-8 value in the non ICU group was >1000 pg/mL then RR 5.2, if the IL-8 value missing in the ICU group is >1000 pg/mL and the IL-8 value in the non ICU group is <1000 pg/mL then RR 12.8.

The IL-6 levels in both groups were extremely low (the cut-off value of IL-6 at 250 pg/ml was not reached [11]). The mean in the ICU group was 108 pg/mL and in the non-ICU group 32 pg/mL. In non-neutropenic patients with sepsis the values of IL-6 are at least 1000-10,000 fold higher. Because of these extreme low values of IL-6 statistics on this parameter was not allowed. IL-8 levels measured on day 1 correlated most significantly with outcome (Fig 3). On day 1, the median value in the ICU group was 2882 pg/mL (IQR 1228-4536 pg/mL) and the median in the non-ICU group was 146 pg/ml (IQR 66.7-213 pg/mL) (Fig 3; p=0.001). Two data points are missing; one in each group. IL-8 was not measured at that time-point. Using a cut-off value of 1000 pg/mL (Ref 10 ROC cut-off 980 pg/mL) 6 out of 7 patients were found to have a high IL-8 in the ICU group compared to 2 out of 16 patients in the non-ICU group. The risk of ICU admittance was 11.3 times ([95% CI 1.6-77.9]) higher in the elevated IL-8 group than in the non-ICU group. All 3 patients who died had IL-8 >1000 pg/mL.

The longitudinal data showed a decrease in level of IL-8 and CRP, but not a normalization within 48 hours (see Table 3). All other inflammatory markers were not significantly different between both groups. Values of IL-10 were extremely low (<15 pg/mL) and did not show any correlation with outcome. It was noted that there was no rise of TNF-α in any patient at any time-point (<5 pg/mL).

### Discussion

The annual incidence of enterocolitis in our single pediatric oncology unit is estimated at 4-6% of newly diagnosed patients over this 3 year period. In review articles the annual incidence ranges from 12-46% [17-19]. Of the included patients only 16% had a C. difficile positive-colitis. The carriage rate of C. difficile in oncology patients is 15-30%. 70% of these patients will show signs of clinical illness [20,21]. The low percentage of Clostridium-positive colitis in our series can be explained by the fact that 19 out of 25 patients (76%) were pretreated with antibiotics of which 13 patients (52%) had received vancomycin prior to rectoscopy (Fig.1). However in this group 5 patients (38%) still showed pathological changes by rectoscopy, which indicates that these patients may develop colitis in spite of adequate antibiotics. This illustrates that a variety of factors are involved in causing and modulating mucosal barrier injury, including
Pseudomembranous and neutropenic enterocolitis in pediatric oncology patients

the chemotherapy given, the elaboration of pro-inflammatory and other cytokines, such as endotoxins across the mucosal barrier, translocation of the resident microflora and their products, and the exposure to antimicrobial agents [8].

In the older literature the severe enterocolitis was mainly seen in hematological malignancies [22,23], but with the general intensification of chemotherapy it has been more common to find these enterocolitis entities in both solid and hematological malignancies [24]. These findings were confirmed in this study: 44% of the patients had a hematological malignancy and 52% had a solid tumor.

Microbiological studies revealed bacteremia in 24% of the patients: of these cultures 57% showed growth of Gram-positive organisms, 29% Gram-negative organisms, and 14% fungal organisms. Katz et al [23] reported bacteremia in 84% of patients and fungemia in 16%. One possible explanation for a lower incidence of bacteremia in this unit is that all patients were started on selective decontamination of the intestinal tract shortly after chemotherapy. Another explanation might be that with special techniques to detect cell-wall deficient strains more (about 5%) episodes of bacteremia might be detected [25].

The main aim of the study was to identify patients likely to develop a severe enterocolitis. The clinical symptoms diarrhea, abdominal pain and, mucositis showed no significant difference between the ICU and the non-ICU group. This is also described in the literature [7]. The pattern of fever has low prognostic value in the sense that the ICU group had more ‘long and continuous fever’ than the non-ICU group.

The rectal biopsy findings did not contribute to treatment decisions, it was shown that the findings of pseudomembranes on rectal biopsy correlated fully with C. difficile toxin positivity or culture positivity. None of the patients had any complications after the procedure and all patients did not experience the procedure as painful. Because the yield was so low we would not recommend to perform this procedure in future trials, although it might warrant to look at inflammatory parameters in rectal dialysate instead. Routine laboratory investigations could not distinguish the severe cases from the less severe cases. Sloas et al [26] who investigated 24 confirmed cases of typhlitis retrospectively also reported that routine laboratory investigations were not informative. Imaging was not included in the study. Most of the patients had an abdominal ultrasound performed on day one and in most of these ultrasound widened bowel-loops were found and thickened bowel wall. However this was not scored accurately or prospectively validated therefore this was not included as outcome measure, this should be considered in future trials as bowel wall thickening is becoming a diagnostic tool [27,28].

The inflammatory parameters in the present study showed that CRP and IL-8 were of prognostic value. The extent and course of serum concentrations of IL-8 and IL-6 in the patients with colitis symptoms were significantly different from those seen in septic conditions in non-neutropenic children [29]. In non-neutropenic patients concentrations of IL-8 are at least 10-100 fold higher, and the IL-6 levels are at least 100-1000 fold higher [29]. IL-8 used at a cut-off point of 1000
pg/mL, was found to be a strong prognostic factor. Although decreasing over time, the IL-8 levels in our patient cohort did not normalize within 24-48 hours after administering intensive care treatment and support as is usually observed in children with non-neutropenic sepsis. Although the chemotactic activity towards neutrophils is the most important function of IL-8, we know that in these patients neutrophils are missing and also absent in the extravascular tissues. Taken together, these data indicate that IL-8 in neutropenic patients is less likely derived from enterocytes and myeloid cells, but from tissue cells such as endothelial cells, and fibroblasts [15,30]. The chronic low flow and hypoxia prone situation is not impossible as a contributing factor for endothelial cell-induced IL-8 generation. This might be mediated by hypoxia-sensitive AP-1 and NF-kB-like binding sites in the IL-8 promoter site. In such patients, a role for IL-8 as an angiogenesis-regulating factor may be more important. Strikingly the IL-6 values were found to be low. The role of IL-6 remains unclear, although IL-6 is a multifunctional cytokine regulating B and T cell function and the acute phase response [31], neither prolonged fever nor CRP levels correlated with serum IL-6.

With this possible pathophysiological mechanism in mind, we hypothesize that the IL-8 increase seen in our patients reflects the extent of damage of the intestinal wall, and could therefore predict the severity of the disease. For the daily clinical practice, we would strongly advise to initiate early aggressive supportive care in neutropenic patients with abdominal cramps and diarrhea in whom IL-8 levels are elevated, of course, the measurement of such inflammatory parameters may very much depend on the assay used and will require further standardization. The supportive care thought of would be starting inotropic support on the first day like dopamine at a dose of 5 \( \mu g/\text{kg/min} \) to increase the gastro-intestinal blood-flow. Future intervention trials should concentrate on the use of granulocyte-transfusions, enterocyte-healing factors such as EGF and anti-inflammatory factors.

Acknowledgment

We are grateful to our pediatric gastroenterology department for performing the rectoscopies and mucosal biopsies, to our pathology department doing the microscopy, and to our clinical and epidemiological biostatistics department for their statistical advice.

References


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17. Moir DH, Turner JJ, Ma DD, Biggs JC. Autopsy findings in bone marrow transplantation. Pathology 1982, 14, 197-204.


Chapter 5

Prophylactic antibiotics for preventing early Gram-positive central venous catheter infections in oncology patients, a Cochrane systematic review

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Submitted

(shortened version of Cochrane review: Cochrane library: issue 2: 2003
see addendum)
Abstract

**Background:** Long-term tunnelled central venous catheters (TCVC) are increasingly used in oncology patients. Despite international guidelines on sterile insertion, appropriate catheter maintenance and use, infections are a frequent complication of TCVC. These infections are mostly caused by Gram-positive bacteria. The aim of this review is to evaluate the efficacy of antibiotics in the prevention of early TCVC infections.

**Methods:** We searched MEDLINE, EMBASE, and the Cochrane Controlled Trials Register up to July 2003. Reference lists from relevant articles were scanned and conference proceedings were hand searched. **Outcome parameters:** the main outcome was documented Gram-positive bacteremia in patients with a TCVC. **Selection criteria:** randomized controlled trials (RCT) evaluating prophylactic antibiotics prior to insertion of the TCVC, and RCT evaluating the combination of an antibiotic and heparin to flush the TCVC. Both pediatric and adult oncology patient trials were selected. **Data collection & analysis:** the trials identified were assessed and the data extracted independently by two reviewers and a quality assessment was carried out.

**Main results:** We included 9 trials with a total of 529 patients. Four trials reported on vancomycin/teicoplanin prior to insertion of the TCVC compared to no antibiotics. Five trials studied flushing of the TCVC with a vancomycin/heparin solution compared to heparin flushing only. Antibiotics prior to insertion of the catheter compared to no antibiotics showed a significant reduction in the number of Gram-positive TCVC infections with an Odds ratio of 0.46 (95% confidence interval 0.24-0.91). Flushing the TCVC with antibiotics and heparin compared to only heparin significantly decreased the number of TCVC infections with an Odds ratio of 0.43 (95% CI 0.21-0.87).

**Conclusion:** Both interventions (antibiotics prior to insertion of the catheter and flushing of the catheter with the combination of an antibiotic and heparin) significantly reduced the incidence of Gram-positive infections in TCVC. In oncology patients who need a TCVC and are at high risk for Gram-positive infections it is justified to use the above interventions.
Prophylactic antibiotics for preventing early Gram-positive central venous catheter infections in oncology patients

Introduction

Patients treated for cancer need adequate venous access because of the frequent use of chemotherapy, requirements of intravenous fluids and blood products. To limit discomfort of short-term venous access long-term tunneled central venous catheters (TCVC) are used in more than two thirds of both pediatric and adult cancer patients \(^{1\text{-}3}\). However, the use of TCVC is limited by the risk of blood clot formation as well as infectious complications. The risk of infection ranges from 1.4\(^\text{d}\) to 2.2\(^1\text{l}\) infections per 1000 catheter days. This corresponds to about one third of patients experiencing an episode of infection while having the TCVC in place\(^5\). The risk of infection is greatest during the first 100 days after placement of the TCVC \(^6\). In the majority of the cases the causative agents are Gram-positive organisms (70%), followed by Gram-negative organisms (15%), and fungi or anaerobic organisms (both 7%).

Early catheter infections (defined as infections that develop within 45 days after placement of the catheter) are mostly due to organisms from the skin at the insertion site, whereas after 45 days the catheter hub becomes a far more important source of infection \(^7\). The adherence to and colonization of TCVC with micro-organisms is facilitated by the formation of a very thin biofilm inside the catheter lumen. This process is influenced by several factors, such as the production of fibroglycocalyx (extracellular slime) by coagulase negative staphylococci. Despite international guidelines on catheter insertion and handling, developed by the Hospital Infection Control Practices Advisory committee,\(^5\text{-}10\) about 15-20% of patients with a TCVC develop early infections. It has been shown that the incidence of Gram-positive infections is increasing, mainly coagulase negative staphylococci.\(^11\). Therefore, prophylactic antibiotics covering Gram-positive organisms might decrease catheter-related infections. Antibiotics can be introduced for this goal in two ways, either prior to the insertion of the catheter, or by flushing the catheter with a combination of an antibiotic and heparin during the life-span of the catheter.

Several trials have evaluated the beneficial effects of these strategies, however results have been conflicting. Therefore we performed a Cochrane systematic review, to assess the effectiveness of prophylactic antibiotics to reduce early Gram-positive catheter related infections. The importance of this time-span allows us to cover the induction period of chemotherapy. The time-period that many manipulations of the TCVC are necessary because of the intensity of the chemotherapy. Both internal and external tunnelled central venous catheters could be included in the review.

Methods

Search

A literature search was performed using MEDLINE and EMBASE from 1966-2003 and the CENTRAL(Cochrane library) issue 2, 2003. We used the following terms:
Broviac OR Port-a-cath OR Port OR Portacath OR Hickman OR exp catheterization,central venous OR Tunnelled central venous catheter
AND:
prophylactic antibiotics OR vancomycin OR teicoplanin OR antibiotics
AND
exp oncology OR cancer OR malignancy OR neoplasm OR leuk(a)emia OR carcinoma
AND
infection.exp OR infection prevention OR infection control OR bacter(a)emia OR sepsis
AND
The sensitive methodology filter of the Cochrane handbook for RCT
Additional trials not registered in MEDLINE or the Cochrane Library were identified by scanning the reference lists of the found articles.

Inclusion and exclusion criteria
We included all randomized controlled trials (RCTs) that compared antibiotics to no antibiotics prior to insertion of the catheter, and all RCT that compared the antibiotic/heparin flush technique to flushing with heparin only to reduce Gram-positive infections related to the catheter. Trials were included considering oncology patients (both adults and children) undergoing chemotherapy who had a tunneled central venous catheter inserted, and who were likely to become neutropenic. In reviewing the papers both adults and children were included, as the adult patient is comparable to the pediatric patient in the development of TCVC infections. We excluded trials if patients had an infection prior to insertion of the catheter, or if they received continuous intravenous antibiotics before and after insertion of the catheter.

Data-extraction
Two reviewers (MvdW, JWW) independently abstracted the following data: year of publication, characteristics of the patients, including age and type of malignancy, type of catheter inserted, number of patients in each arm. Life-span of the catheter, number of catheter related infections, number of catheter related bacteremia's, time to first catheter related infection, culture method (quantitative or qualitative), type of organism cultured (Gram-positive, Gram-negative, fungal), outcome of the infection that was defined as improvement of the patient and clearing of the organism on antibiotics only or removing the catheter. Definitions used for catheter related infections were defined according to the Hospital Infection Control guidelines \[2\] i.e. isolation of
the same organism from percutaneous blood culture and from one of the following: exudate at the catheter exit site, a semiquantitative catheter segment culture (requiring removal of the catheter), or a blood-culture from the TCVC lumen with a recovery of at least five fold higher colony count than from the peripheral blood. Catheter-related infection could also be defined when there was a temporal succession of catheter flushing, onset of chills, fever and a positive blood culture, or if the culture from the TCVC was positive 2 hours earlier than the culture from the peripheral blood, this method is called differential time to positivity and makes use of continuous blood-culture monitoring. A tunnel infection was defined if a spreading cellulitis was present over the tunnel tract of the subcutaneous tunnelled catheter. There are signs of inflammation and tenderness to palpation over the tunnel tract.

Disagreements were resolved between the 2 reviewers by discussion.

**Quality assessment**
The quality-list of Tulder 13 was used. The Tulder criteria consist of 17 items assessing both internal and external validity, including allocation generation and concealment, blinding of the patient and of the outcome-assessor, method of analysis (intention to treat), number of drop-outs, follow-up including the end-point of the study and description of the statistics.

Outcome and analyses:
The primary outcome was bacteremia due to central venous catheter infections. We measured the number of patients with catheter-related bacteremia compared to the total number of patients. If necessary these data were extracted from the raw data. The secondary outcome was the number of tunnel-infections reported. The trials were divided into two groups which were analysed separately.

1: prophylaxis of antibiotics at insertion of the central venous catheter versus no prophylaxis
2: vancomycin/heparin flush technique versus only heparin flush technique

The analyses performed were:

1: The number of patients with catheter-related bacteremia in the group who received antibiotics prior to insertion compared to the number of patients with catheter-related bacteremia in the group who did not receive antibiotics.

2: The number of patients with catheter-related bacteremia in the group flushing the catheter with a vancomycin/heparin solution compared to the number of patients with catheter-related bacteremia in the group flushing the catheter only with heparin.

**Statistics**

Dichotomous data were analyzed calculating the Odds ratio for each trial with the uncertainty in each result being expressed using 95% confidence intervals (CI). A standard meta-analysis was performed where all individual trials were weighted by the inverse of the variance. (The software used was The Cochrane Reviewmanager 4.1) Throughout the review a fixed effect
model was used. Trials were pooled if there was clinical homogeneity, in therapy given and outcome looked at. A random effect model was used if the included trials were heterogenous in design, intervention and study population. Heterogeneity (degree of difference between the results of different trials) was calculated with the chi square test of heterogeneity. The outcome considered was the number of patients with catheter-related bacteremia compared to the total number of patients. Pooling was only considered if ≥ 3 trials were present in the same group.

Results
We scanned the abstracts of 33 articles retrieved from the literature search. A total of 14 trials were evaluated for inclusion, of which 5 were excluded (Table 3). Three trials were excluded because the results were analysed retrospectively[14-16] and 2 because non-tunnelled catheters were studied[17,18] (Table 3). Of the 9 trials included, 4 addressed the administration of antibiotics prior to insertion of the catheter (Table 1)[11,19,21], and 5 addressed flushing the catheter with the combination of vancomycin and heparin (Table 2)[22-26]. In total 588 patients were included in the 9 trials. Four trials were done in adults (n=252)[11,19,21, three in children (n=192)[24-26], and two combined children and adults (n=144)[22,23]. In all trials single or double lumen external tunnelled central venous catheters were used. Seven trials included both patients with solid tumours and hematological malignancies and in 2 trials only patients with hematological malignancies were included[11,19]. In 7 trials qualitative culture methods were used to define catheter related bacteremia, in 2 quantitative methods[24,26]. No additional trials were obtained from the conference proceedings.

Quality-assessment
In 5 trials the method of blinding was adequately described, whereas in 4 trials investigators were not adequately blinded[11,19,21,25] (Table 1 and 2). In 6 trials randomization procedures were adequate, in 3 semi-randomization methods were used. In one these patients were initially randomised to vancomycin treatment or not, but later all patients received treatment[21]. Therefore we only used the first part of the study (i.e. when randomization was performed) in our analysis. In the second study an open randomization was performed and the study was stopped at interim analysis[19]. The third study did not specify how patients were randomized[11]. In one study no intention to treat analysis was applied[20].

Outcome and analysis
Intravenous antibiotics at TCVC insertion
Of the 4 trials that evaluated the efficacy of antibiotics prior to the insertion of the TCVC, 2 studied teicoplanin[11,19] and 2 studied vancomycin before insertion of the catheter[11,19]. Since we aimed to review the prevention of Gram-positive TCVC infections and both vancomycin and
teicoplanin are glycopeptides, that are equally active against Gram-positive bacteria we felt it was acceptable to pool the data of these 4 trials. In all trials the number of patients, the number of TCVC's inserted as well as the number of catheter-related bacteremia's in the treatment group and the control group were given. The follow-up time of all trials did not exceed 30 days. Since there was heterogeneity in the patient groups of the trials (p=0.06) mainly caused by the study of Ljungman et al. who studied a large group of allogenic bone-marrow transplant patients, who were already given TMP/SMZ (trimethoprim/sulphamethoxazole) one week prior to insertion of the catheter, we analysed the pooled data after exclusion of this study. This resulted in a more homogenous group of trials with a p-value for test of heterogeneity of 0.11. There were 17 patients with a Gram-positive bacteremia in the group who received prophylaxis (n=95) and in the control group there were 30 patients with a Gram-positive bacteremia (n=92). There was a significant benefit for the use of antibiotics prior to insertion of the catheter. The pooled OR was 0.46 (95% CI 0.24-0.91) (Fig 1).

No analysis on decreasing tunnel-infections was done, since only 2 trials reported on this item. In the first study by Ranson et al. patients were stratified in hematological patients and patients with solid tumours. In the first group vancomycin resulted in a reduction of 10% in the occurrence of tunnel-infections, whereas in the latter group no tunnel-infections occurred. In the second trial trial of Lim the same trend was observed, with an absolute reduction of 7%.

**Figure 1:** Pooled data of the three trials administering antibiotics prior to insertion of the catheter. The number of patients in the treatment group is presented with a Gram-positive catheter related infection and the number of patients with a catheter related infection in the control-group is given. The OR and 95% CI are represented.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranson 1990</td>
<td>9/36</td>
<td>9/36</td>
<td>26.58 1.00 [0.34, 2.91]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lim 1993</td>
<td>7/43</td>
<td>16/45</td>
<td>51.55 0.35 [0.13, 0.97]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vassilomaniakis 1995</td>
<td>1/16</td>
<td>5/11</td>
<td>21.88 0.08 [0.01, 0.84]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>95</td>
<td>92</td>
<td>100.00 0.46 [0.24, 0.91]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 17 (Treatment), 30 (Control)

Test for heterogeneity: Chi² = 4.43, df = 2 (P = 0.11)

Test for overall effect: Z = 2.23 (P = 0.03)
### Table 1: Trials using antibiotics prior to insertion of the catheter

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Patients</th>
<th>Intervention</th>
<th>Allocation Concealment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lim 1993&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Adult oncology patients &lt;br&gt;Hematological disorders (n=88)</td>
<td>Teicoplanin 400 mg within 2 hours before insertion catheter versus control</td>
<td>B</td>
</tr>
<tr>
<td>Ljungman 1997&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Adult oncology patients BMT and leukaemia (n=66)</td>
<td>Teicoplanin 400 mg within 2 hours before insertion catheter and 24 hours after insertion versus control</td>
<td>B</td>
</tr>
<tr>
<td>Ranson 1990&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Adult oncology patients 2 groups &lt;br&gt;(n=48) acute leukaemia and BMT &lt;br&gt;(n=50) solid tumour pts</td>
<td>Vancomycin 500 mg 30 min prior to insertion and 500 mg 2 hours after insertion versus placebo</td>
<td>A</td>
</tr>
<tr>
<td>Vassilomaniakis 1995&lt;sup&gt;21&lt;/sup&gt;</td>
<td>Adult oncology patients (n=30) &lt;br&gt;solid tumours, (n=10) leukemia/lymphoma</td>
<td>Vancomycin 500 mg within 1 hour prior to insertion catheter and 500 mg 6 and 12 hrs later versus control</td>
<td>B</td>
</tr>
</tbody>
</table>

### Table 2: Trials using the vancomycin/heparin flush technique

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Patients</th>
<th>Intervention</th>
<th>Allocation Concealment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barriga 1997&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Adult and pediatric pts &lt;br&gt;Mainly leukaemia (n=83)</td>
<td>Daily flushing of the catheter with 5 ml V/H solution (vanco 25 mcg/ml, hep 25U/ml) versus only H solution</td>
<td>A</td>
</tr>
<tr>
<td>Daghistani 1996&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Adult and pediatric pts &lt;br&gt;Mainly leukaemia (n=61)</td>
<td>Daily flushing of the catheter with 5 ml V/A/H (vanco 25 mcg/ml, hep 100 U/ml, amikin 25mcg/ml) solution versus only H solution</td>
<td>A</td>
</tr>
<tr>
<td>Henrickson 2000&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Pediatric oncology pts Leukaemia/ BMT and solid tumours(n=126)</td>
<td>Daily flushing of the catheter with 5 ml V/H solution (vanco 25 mcg/ml, hep 10 U/ml) versus only H solution</td>
<td>A</td>
</tr>
<tr>
<td>Rackoff 1995&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Pediatric oncology patients, leukaemia, lymphoma and neuroblastoma (n=55)</td>
<td>Daily flushing of the catheter with 3 mls V/H solution (vanco 25 mcg/ml, hep 100 U/ml) versus only H solution</td>
<td>A</td>
</tr>
<tr>
<td>Schwartz 1990&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Pediatric oncology patients &lt;br&gt;mainly leukaemia (n=45)</td>
<td>Daily flushing of the catheter with 5 mls V/H solution (vanco 25 mcg/ml, hep 10 U/ml) versus only H solution</td>
<td>A</td>
</tr>
</tbody>
</table>

**Heparin/vancomycin flush technique after CVC insertion**

The data of the 5 trials using vancomycin/heparin flush method compared to only heparin flush method (control) were pooled. All these trials were done in pediatric oncology patients. In this group of trials the follow-up time was longer than in the previous group. The flush method was used throughout the life-span of the catheter and, the mean time the CVC was inserted was 250 days per patient in all trials. There was no heterogeneity in the patient groups included.
Prophylactic antibiotics for preventing early Gram-positive central venous catheter infections in oncology patients

<table>
<thead>
<tr>
<th>Blinding</th>
<th>Intention to treat</th>
<th>Treatment Group Gram+CRS</th>
<th>Control group Gram+CRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>A</td>
<td>7/43</td>
<td>16/45</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>2/33</td>
<td>0/32</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>9/36</td>
<td>9/36</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>1/16</td>
<td>5/11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blinding</th>
<th>Intention to treat</th>
<th>Treatment Group Gram+CRS</th>
<th>Control group Gram+CRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>7/39</td>
<td>16/44</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>3/34</td>
<td>2/30</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>1/28</td>
<td>7/64</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>2/28</td>
<td>1/27</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>0/21</td>
<td>6/24</td>
</tr>
</tbody>
</table>

(p=0.32). In the patients who received flushing of the catheter with an antibiotic and heparin there were 13 patients with a Gram-positive bacteremia (n=150) and in the control group there were 32 patients with a Gram-positive bacteremia (n=189). Using the fixed model this resulted in a significant reduction of Gram-positive catheter related bacteremia's using the flush method. An OR of 0.43 (95% CI 0.21-0.87) was found (Fig 2).

As it is of interest to know the catheter related infection rate in the first 30 days after insertion
Table 3: Excluded trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Sibai 1987</td>
<td>146 patients with malignant disease received 160 Hickman catheters</td>
<td>70 patients received prophylactic antibiotics during and after insertion of the catheter, 91.5% of these pts received vancomycin. catheter-infection-rate dropped from 0.50 to 0.25 per 100 days</td>
</tr>
<tr>
<td>Dawson 2000</td>
<td>143 pediatric oncology patients, 176 TCVC</td>
<td>Intervention: Cephalothin 100 mg/kg iv or vancomycin 20-25mg/kg prior to insertion. Rate of infections &lt;30 days dropped 40%</td>
</tr>
<tr>
<td>Rubie 1994</td>
<td>163 pediatric patients with cancer had 180 subcutaneous ports inserted</td>
<td>Because of increased CNS catheter related infections care and maintenance of the ports was changed from only flushing with heparin to a V/H solution (50 mcg/ml V and 100U/ml H). The staphylococcus sepsis rate dropped from 31% to 4%</td>
</tr>
<tr>
<td>Carratella 1999</td>
<td>120 adult oncology patients mainly leukaemia patients who had non-tunnelled catheters inserted</td>
<td>When patients were neutropenic the treatment group received a V/H solution (25 mcg/ml V, 10U/ml H 2.5 ml) dwell for 1 hour every second day. Control group received only heparin solution The catheter related sepsis rate dropped from 7% to 0%</td>
</tr>
<tr>
<td>Raad 1998</td>
<td>26 patients with melanoma on IL-2 treatment received non-tunnelled catheters</td>
<td>Prophylactic antibiotics given were novobiocin 500 mg + rifampin 300 mg. Catheter related bacteremia decreased from 41% to 6%</td>
</tr>
</tbody>
</table>

Figure 2: Pooled data of the five trials flushing the catheter with an antibiotic and heparin. The number of patients in the treatment group is presented with a Gram-positive catheter related infection and the number of patients with a catheter related infection in the control-group is given. The OR and 95% CI are represented.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwartz 1990</td>
<td>0/21</td>
<td>6/24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rackoff 1995</td>
<td>2/28</td>
<td>1/27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daghistani 1996</td>
<td>3/34</td>
<td>2/30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barriga 1997</td>
<td>7/39</td>
<td>16/44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Henrickson 2000</td>
<td>1/28</td>
<td>7/64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>150</td>
<td>189</td>
<td></td>
<td>100.00</td>
<td>0.43[0.21,0.87]</td>
</tr>
<tr>
<td>Total events: 13 (Treatment), 32 (Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 4.67, df = 4 (P = 0.32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 2.36 (P = 0.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of the catheter we attempted to extract these data from the raw data. Only two trials allowed us to deduct these figures from the Kaplan-Mayer curves given. Schwartz et al 26 showed 1 bacteremic episode in the control-group, none in the treatment group in the first 50 days. In addition Henrickson et al 24 showed 7 bacteremic catheter related episodes in the control-group compared to one in the treatment group.
<table>
<thead>
<tr>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic use and duration at the discretion of the attending physician, results retrospectively analyzed.</td>
</tr>
<tr>
<td>No randomization done. Intervention period was compared to pre-intervention period</td>
</tr>
<tr>
<td>Not randomized. A change of policy over time Results retrospectively analyzed.</td>
</tr>
<tr>
<td>Non-tunneled catheters used And a flush solution that was allowed to dwell</td>
</tr>
<tr>
<td>Non-tunneled catheters and the antibiotics were administered within 48 hours of receiving the central line, not before insertion</td>
</tr>
</tbody>
</table>

**Discussion**

Infections due to Gram-positive organisms in oncology patients have increased over the past years. Colonization of central venous catheters with Gram-positive organisms can occur at the insertion-site and these organisms can become invasive when patients are neutropenic and immune suppressed. Therefore preventive strategies must reduce colonization of the insertion site and decrease spread both extraluminal from the skin and intraluminal from the hub to the catheter tip. The main preventive strategy remains adequate education in care and maintenance of the catheter. Education of parents and care-givers about the handling of the catheters has been shown to reduce catheter-related infections. In addition it has been shown that the number of care takers that manipulates the TCVC should be minimized in order to reduce infectious complications. The most important preventive measure is careful handwashing. To reduce the extraluminal spread of Gram-positive organisms via the insertion-site antibiotic prophylaxis at the time of catheter-placement has been evaluated in a number of trials. Because of controversial results of these trials however, this has not lead to recommendation of the use of prophylactic antibiotics in most guidelines. These guidelines however have been based on heterogeneous patients groups including neonates and patients who are on total parenteral nutrition for other reasons than cancer. In this systematic review we only included trials in oncology patients in need of a tunneled central venous catheter inserted for chemotherapy.
bloodproducts and fluids. Study design and methodology of the 4 included trials were sufficient and of these the data of 3 trials could be pooled. We found a significant reduction (OR =0.46: 95% CI 0.24-0.91) of Gram-positive catheter related infections with the use of vancomycin or teicoplanin before insertion of the catheter. This OR indicates that in a unit with a baseline incidence of tunneled catheter related sepsis (TCRS) that ranges from 10% to 30 % the number needed to treat (NNT) to prevent 1 TCV infection ranges from 10 to18 patients. It is justifiable to administer antibiotics prior to insertion of the catheter in high risk patients. The short prophylaxis will limit the emergence of resistant strains.

To reduce the intraluminal spread of Gram-positive organisms via the hub of the catheter the effect of antibiotic flushing of the catheter was evaluated. The most important step is the care in accessing the hub. The hub should be desinfected before access. More frequent catheter-hub manipulation increases the risk of contamination. Therefore, it is recommended to minimize the number of times per day to handle the catheter-hub. Flush solutions should contain heparin to prevent thrombotic events. The efficacy of adding vancomycin to the flush solution to decrease Gram-positive catheter related infections in oncological patients has not been clearly established so far. In vitro trials have proven that the combination of heparin and vancomycin 25 mcg/ml is stable and active. In the second part of this systematic review we aimed to answer the question if the vancomycin/heparin flush technique can decrease Gram-positive catheter related infections in oncology patients with a tunneled CVC.

Design and methodology of the 5 included trials that have evaluated this were sufficient to pool and analyze the data. We found that vancomycin/heparin flush technique is effective in reducing TCRS (OR 0.43, 95% CI 0.21-0.87) The number needed to treat to prevent 1 catheter-related sepsis is 13 with an infection rate of 30% and 23 with an infection rate of 10%. The fear for resistant strains does not seem justified as the dose of vancomycin is so low that it will not distribute systemically. The possibility exists to develop super infections with Gram-negative organisms and fungal organisms, therefore the advice of the authors is to take the TCRS rate into account before considering to flush the tunneled central venous catheter with an antimicrobial agent.

**Implications for practice**

We feel that a selection of patients with a high base-line risk for infection (i.e. hematologic patients receiving induction chemotherapy, patients who are neutropenic at the time of insertion of the catheter and patients undergoing a bone-marrow transplant) will benefit from the use of antibiotics prior to insertion of the central venous catheter, or flushing the central venous catheter with a combination of an antibiotic and heparin.
Prophylactic antibiotics for preventing early Gram-positive central venous catheter infections in oncology patients

Acknowledgements
This research was supported by a research grant from the SKK (Stichting Kinder-geneeskundig Kanker onderzoek). We would also like to thank Dr R.J.P.M. Scholten, epidemiologist Dutch Cochrane Center for reviewing the manuscript and assisting with the statistical part.

Reference List


Chapter 6

Efficacy of selective decontamination of the digestive tract (SDD) in oncology patients: systematic review of randomized controlled trials

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¹ Department of Pediatric Oncology Emma Children’s Hospital
² Center for Pediatric Clinical Epidemiology Emma Children’s Hospital
³ Dutch Cochrane Center and Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam, The Netherlands

Submitted
Abstract

Introduction: The use of selective decontamination of the digestive tract (SDD) in oncology patients is still a matter of debate. A systematic review was performed to assess the evidence for the effectiveness of SDD to decrease bacteremia and infection-related mortality in oncology patients during neutropenic episodes.

Methods: We searched Medline from 1966 to October 2002, Embase from 1988 to 2002 and the Cochrane Register of Controlled Trials issue 2, 2002. The search was supplemented by checking the references of these articles for additional papers. The main outcome was the number of patients with documented bacteremia (Gram-negative or Gram-positive bacteremia) and infection related mortality. For the trials meeting the inclusion-criteria data-extraction and quality assessment were performed independently by two reviewers.

Main results: A total of 21 trials met the inclusion criteria. Seventeen trials compared SDD (quinolones or Trimethoprim/sulfamethoxazole (TMP/SMZ)) to no SDD, and 4 trials compared quinolones to TMP/SMZ. The incidence of Gram-negative bacteremia decreased significantly (pooled Odds Ratio 0.34, 95% CI 0.21-0.54) without an increase in Gram-positive bacteremia. This effect was seen for TMP/SMZ and for quinolone based SDD regimens. Quinolone-based regimens yielded a stronger reduction in Gram-negative bacteremia, and TMP/SMZ based regimens a stronger reduction in Gram-positive bacteremia. Infection related mortality due to bacterial causes decreased with the use of SDD (pooled OR 0.49, 95% CI 0.27-0.88).

Conclusion: SDD decreases Gram-negative bacteremia and infection related mortality due to bacterial causes during neutropenic episodes in oncology patients without increasing Gram-positive bacteremia.
Efficacy of selective decontamination of the digestive tract (SDD) in oncology patients

Introduction

Complications due to infection remain a source of morbidity and mortality in oncology patients. The infection mortality rate is around 4-6% in adult patients and 0.4-1.0% in pediatric patients. With the intensified treatment of both solid and hematological malignancies, episodes of severe neutropenia are induced by cytotoxic chemotherapy, leading to an increased chance of infectious complications. Therefore, prevention of infections is of importance in this group of patients. Mortality due to infections is much higher for Gram-negative bacteremia than for Gram-positive bacteremia. Therefore, special effort should be focused to reduce infections with Gram-negative organisms.

In the 1970's, van der Waaij et al. developed a strategy to reduce the frequency of infections especially Gram-negative bacteria in the immuno-compromised patient. By this strategy, named selective decontamination of the digestive tract or SDD, potentially pathogenic aerobic microorganisms are eliminated from the gastro-intestinal tract, without affecting the non-pathogenic anaerobic flora. SDD is achieved by administration of oral partly absorbable and partly non-absorbable antibiotics, often in combination with anti-fungal prophylaxis. For adequate use of SDD it is essential that the oral antibiotics are given before the start of neutropenia to achieve optimal eradication of the potential pathogenic aerobic micro-organisms in the digestive tract. Several randomized controlled trials have been conducted to assess the question whether prophylaxis with Trimethoprim/sulfamethoxazole (TMP/SMZ) was a successful strategy to reduce the frequency of Gram-negative infections in neutropenic patients. TMP/SMZ destroys aerobic Gram-negative bacteria in the colon without affecting the anaerobic flora. TMP/SMZ significantly reduced the rate of bacterial infections. Despite the reported successes of TMP/SMZ, several disadvantages were associated with its use, including hypersensitivity to the drugs, prolongation of the duration of neutropenia and the occurrence of infections with resistant Gram-negative bacteria.

In the 80's, fluoroquinolones were considered more promising drugs for SDD. These antibiotics had an increased activity against Gram-negative bacteria. Furthermore, they were not as myelosuppressive as TMP/SMZ and they were not associated with hypersensitivity. Many randomized controlled trials have been performed, assessing the effectiveness of fluoroquinolones in SDD, and are summarized in two systematic reviews. Although these trials show an important reduction in the incidence of Gram-negative infection, no reduction was found in infection-related mortality.

Despite the amount of trials involving SDD, there is no consensus whether SDD reduces infection-related mortality and whether SDD should be given or not given to patients at risk. Our goal in this systematic review is to evaluate the efficacy of the use of oral SDD (TMP/SMZ or quinolones) started before the expected onset of neutropenia in decreasing bacteremia and infection-related mortality in neutropenic oncology patients, and defining a subgroup of patients who will benefit most from the use of this strategy.
Methods

Search

A literature search was done using Medline from 1966 to October 2002, Embase from 1988 till October 2002 and the Cochrane Central Register of controlled trials issue 2, 2002. We used the following terms:

prevention OR infection control OR selective decontamination OR decontamination digestive tract OR prophylactic antibiotics OR framycetin OR colistin OR nystatin OR neomycin OR trimethoprim-sulfamethoxazole OR trimethoprim OR sulfamethoxazole OR co-trimoxazole OR nalidixic acid OR quinolone OR fluoroquinolone OR norfloxacin OR ciprofloxacin OR ofloxacin OR non-absorbable antibiotics OR rifampin OR roxithromycin

AND

Leuk(a)emia OR cancer OR oncol* OR malignan* OR neoplasm* OR antineoplastic OR carcinoma

AND

agranulocytosis OR neutropeni* OR aplasia OR granulocytopeni* OR leukopeni*

AND

randomized controlled trial OR drug therapy OR therapeutic use OR random*(using a sensitive research methodology filter)

We included German, French and English articles. The computer search was supplemented by checking the references of the articles for additional papers.

Inclusion and exclusion criteria

We included randomized trials that compared TMP/SMZ or quinolone based SDD regimens with either placebo or no SDD, and quinolone based SDD regimens compared to TMP/SMZ based regimens. Trials considering oncology patients (both adults and children) undergoing chemotherapy with expected neutropenia and addressing oral SDD (TMP/SMZ or quinolones) that was started before the expected onset of neutropenia were included. We excluded trials using total decontamination of the digestive tract (TDD), trials in which intravenous antibiotics were used as SDD, trials in which infection was present at the time of starting SDD, when SDD was started at the onset of neutropenia, and when SDD was compared to other regimens of SDD, i.e. not TMP/SMZ to quinolones.

Data-extraction and outcome measures

Two reviewers (MvdW, MdW) independently abstracted the following data: year of publication, characteristics of the patients, including age and type of malignancy, type of SDD used, the number of patients included in both treatment arms, the number of neutropenic episodes analysed, the number of patients with bacteremia, and the number of infection related deaths. Disagreements were resolved between the two reviewers by discussion.

We considered as a primary outcome documented bacteremia (Gram-positive or Gram-negative
bacteremia), and mortality (infection related mortality and mortality due to bacterial causes) during febrile neutropenic episodes and as secondary outcome fungaemia and fungal related mortality.

Quality assessment
Two reviewers independently assessed the following criteria regarding the methodological quality of the included trials: allocation concealment (adequate, not adequate, not clear), blinding of patients and outcome-assessors (yes, no, not clear) and the use of an intention to treat analysis (yes, no).

Meta-analysis
Trial specific odds ratios were calculated. Odds ratio’s were combined according to the fixed effect method of Mantel-Haenszel. Trial results were combined if there was clinical homogeneity, in both interventions and outcomes. Statistical heterogeneity was tested with the chi square test of homogeneity. In addition, heterogeneity was quantified by the use of the I² statistic, describing the percentage of total variation across trials that is due to heterogeneity between trials. A value of 0 % indicates no observed heterogeneity between the trials, and 100% indicates only heterogeneity between the trials.

Meta-analyses were performed for the following comparisons: SDD (TMP/SMZ or quinolones) versus placebo or no SDD, and for TMP/SMZ versus quinolones. Subgroup analyses were performed for trials with only conventional chemotherapy patients versus trials with only bone-marrow transplantation patients, and for trials addressing pediatric oncology patients only.

Results
Identification of trials
Our literature search revealed 329 eligible trials (Figure 1). Of those, 294 trials were not included because these were trials on total gut decontamination, trials on using intravenous antibiotics as form of SDD, or fungal prophylaxis, or trials comparing SDD to other combinations of SDD. A total of 36 articles were retrieved. A total of 15 trials did not fulfil the inclusion criteria (see Appendix: Excluded trials). Thus, 21 trials were included. Of these, 17 trials addressed SDD compared to no SDD or placebo and 4 trials compared quinolones to TMP/SMZ (Table 1 and 2).

Quality of the included trials
Of the included 21 trials 13 trials (62%) showed adequate concealment of allocation, in 13 trials (62%) both patient and outcome-assessor were blinded, and 10 trials (47.5%) used an intention to treat analysis. (Table 1 and 2).
Figure 1: All trials included in the search strategy with a breakdown of the articles, which are included for the analysis SDD = selective decontamination of the digestive tract, TMP/SMZ = Trimethoprim/sulfamethoxazole

**Description of included trials**

Seventeen trials compared SDD to placebo or no SDD. Of those, 10 addressed TMP/SMZ and 7 trials addressed quinolones. The 17 trials included 1515 patients and took place from 1983 to 2002. Seven trials were done in patients with hematological malignancies, 3 trials in patients with solid tumors mostly undergoing high dose chemotherapy, 6 trials were done combining hematological and solid tumors, and one trial was performed with only allogenic bone marrow-transplant patients. Three of the trials comparing SDD to no SDD also included pediatric patients (Table 3).

In the control groups (without use of SDD) the incidence of bacteremia ranged from 4.1% (in conventional chemotherapy) to 60% (in allogenic transplant patients), Gram-negative bacteremia from 1.3% to 36% and Gram-positive bacteremia from 2.7% to 38%.

In six of the 17 trials patients used SDD during one neutropenic episode and in 11 trials patients used SDD during multiple neutropenic episodes.

Four trials compared quinolones to TMP/SMZ. Of these, 3 trials included patients with hematological malignancies, and 1 trial included patients with both hematological and...
<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Patients</th>
<th>Intervention</th>
<th>Allocation</th>
<th>Blinding</th>
<th>Intention to treat analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weiser 1981</td>
<td>29 adult patients with leukaemia</td>
<td>TMP/SMZ 960mg 2xd (n=14) vs. placebo (n=15)</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Gualtieri 1983</td>
<td>47 adult patients with leukaemia</td>
<td>TMP/SMZ 1920 mg 1xd (n=24) vs. placebo (n=23)</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Pizzo 1983</td>
<td>150 adults + children (Leukaemia+solid)</td>
<td>TMP/SMZ 10mg/kg in 2 doses + erythro 30mg/kg (n=77) vs. placebo (n=73)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>De Jongh 1983</td>
<td>61 adult patients with small lung cell carcinoma</td>
<td>TMP/SMZ 1040 mg 2xd (n=32) vs. placebo (n=29)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>EORTC 1984</td>
<td>342 adults and children</td>
<td>TMP/SMZ 960 mg 2xd (n=177) vs. placebo (n=165)</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Henry 1984</td>
<td>43 adult patients with leukaemia</td>
<td>TMP/SMZ 960 mg 2xd (n=23) vs. placebo (n=20)</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Kovatch 1985</td>
<td>91 children hematologic and solid tumors</td>
<td>TMP/SMZ (n=43) vs. placebo (n=48)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Ward 1993</td>
<td>42 adult patients leukaemia</td>
<td>TMP/SMZ 960mg 2xd (n=22. placebo (n=20)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Kauffman 1983</td>
<td>55 adult patients with hematologic or solid tumors</td>
<td>TMP/SMZ 480 mg 2xd (n=29) vs. placebo (n=26)</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Kramer 1984</td>
<td>45 adult patients with leukaemia or solid tumors</td>
<td>TMP/SMZ + erythro (29 episodes) vs. placebo (27 episodes)</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Karp 1986</td>
<td>68 adult patients, leukaemia or transplant</td>
<td>Norflox 400 mg 2xd (n=35) vs. placebo (n=33)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Lew 1991</td>
<td>18 adult patients with BMT</td>
<td>Cipro 750 mg 2xd (n=7) vs. placebo (n=11)</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Maiche 1993</td>
<td>59 patients with leukaemia or solid tumors</td>
<td>G-CSF + ofloxacin 200mg 2xd or ciproflox</td>
<td>C</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Talbot 1993</td>
<td>119 adult patients ALL/ANLL</td>
<td>Enoxacin 400mg 2xd (n=62) vs. placebo (n=57)</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Carlson 1997</td>
<td>90 adult patients with ovarian cancer</td>
<td>Cipro 500mg 2xd (n=45) vs. no SDD (n=45).</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Tjøn Heijnen 2001</td>
<td>161 adult patients small lung cell carcinoma</td>
<td>Cipro 750mg 2xd + roxithromycin 150mg 2xd (n=82) vs. placebo (n=79)</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Lee 2002</td>
<td>95 adult patients ANLL</td>
<td>Cipro 250 2xd + rox 150 2xd (n=46) vs. placebo (n=49)</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
</tbody>
</table>

Table 1: Trials comparing SDD (TMP/SMZ or quinolones) to no SDD or placebo. Cipro=Ciproxin, TMP/SMZ= Trimethoprim/sulphamethoxazole, Norflox=Norfloxacin, Erythro=erythromycin, ANLL=acute non-lymphocytic leukaemia, ALL= acute lymphocytic leukaemia, BMT= bone-marrow transplantation. Allocation concealment = A: adequate, B: not adequate C: not clear. Blinding = A: blinding done of patient and outcome assessor, B: blinding not done, C: not clear. Intention to treat analysis= A: yes, B: no.
### Table 2: Trials comparing quinolones to TMP/SMZ. For abbreviations see Table 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Intervention</th>
<th>Bacteremia Bacteremia</th>
<th>Gram-negative Bacteremia</th>
<th>Allocation</th>
<th>Blinding</th>
<th>Intention to treat analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bow 1988</td>
<td>63 adult leukaemia patients</td>
<td>Norflox 400 mg 2xdd TMP/SMZ 960 2xdd</td>
<td>9/31 vs. 6/32</td>
<td>0/31 vs. 4/32</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Dekker 1987</td>
<td>65 adult leukaemia patients</td>
<td>Cipro 500 mg 2xdd vs. TMP/SMZ 960+colistine</td>
<td>7/26 vs. 4/26</td>
<td>4/26 vs. 3/26</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Donnelly 1992</td>
<td>230 adult leukaemia patients</td>
<td>Cipro 500 mg 2xdd vs. TMP/SMZ 960 mg</td>
<td>16/117 vs. 12/113</td>
<td>1/117 vs. 6/113</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Lew 1995</td>
<td>167 adult patients all malignancies</td>
<td>Cipro 750 mg 2xdd vs. TMP/SMZ 960 2xdd</td>
<td>10/75 vs. 6/71</td>
<td>0/75 vs. 1/71</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

### Table 3: Trials on the use of SDD in pediatric patients. For abbreviations see Table 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Intervention</th>
<th>Bacteremia Bacteremia</th>
<th>Gram-negative Bacteremia</th>
<th>Allocation concealment</th>
<th>Blinding</th>
<th>Intention to treat analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>EORTC 1984</td>
<td>N=65 (if only children included)</td>
<td>TMP/SMZ 150/750 mg/m² vs. placebo</td>
<td>2/33 vs. 2/32</td>
<td>?</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Kovatch 1985</td>
<td>N=81 All malignancies</td>
<td>TMP/SMZ 6 mg/kg vs. placebo</td>
<td>1/43 vs. 7/48</td>
<td>0/43 vs. 3/48</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Pizzo 1983</td>
<td>N=150 Leukaemia + solid (adult + child)</td>
<td>TMP/SMZ 10mg/kg + erythro 30mg/kg vs. placebo</td>
<td>3/77 vs. 3/73</td>
<td>1/77 vs. 1/73</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>
solid tumors. All trials included patients who used SDD during multiple neutropenic episodes. A total of 525 patients were included in these latter studies.

Meta-analyses

TMP/SMZ or quinolones versus no SDD

This analysis included 17 trials. Not all articles allowed data-extraction for all end points. The characteristics of the included articles are shown in Table 1 and Table 2. Of the 17 included trials 5 addressed the number of bacteremic episodes compared to the total number of episodes and 12 trials addressed the number of patients with bacteremia compared to the total number of patients.

Effect on bacteremia

Data on bacteremia could be extracted from 12 trials. The 12 trials included 1142 patients. There were 72 patients with bacteremia in the SDD group (n=576) and 124 patients with bacteremia in the control group (n=566). The meta-analysis showed a benefit in favour of SDD use (odds ratio (OR) 0.47; 95% CI: 0.34-0.65). No heterogeneity between the trials was detected (chi square for heterogeneity p=0.74, I²=0%). Of these 12 trials 7 trials used TMP/SMZ as SDD regimen. In these trials 744 patients were included. There were 38 patients with bacteremia in the TMP/SMZ group (n=377) and 64 patients with bacteremia in the control group (n=367). A benefit was shown for the use of TMP/SMZ. The OR was 0.51 (95% CI 0.33-0.79), and there was no heterogeneity between the trials (chi square for heterogeneity p=0.8, I²=0%). Five trials used quinolones as SDD regimen. There were 34 patients with bacteremia in the quinolone group (n=199) and 60 patients with bacteremia in the control group (n=199). A benefit was shown for the use of quinolones. The OR was 0.42 (95% CI 0.25-0.70). There was some heterogeneity between the trials, (chi square for heterogeneity p=0.36, I²=8.8%), but we considered pooling the data useful (Figure 2).

Effect on Gram-negative bacteremia

Data on Gram-negative bacteremia could be extracted from 11 trials. A total of 1047 patients were included. There were 25 patients with Gram-negative bacteremia in the SDD group (n=530) and 59 patients in the group not receiving SDD (n=517). There was a clear benefit shown for the use of SDD. The OR found was 0.39 (95% CI 0.24-0.63). Moderate heterogeneity was seen between the trials but pooling was allowed (chi square for heterogeneity p=0.32, I²=13.5%). Seven trials used TMP/SMZ as SDD regimen. There were 20 patients with a Gram-negative bacteremia in the TMP/SMZ group (n=377), and 29 patients in the control group (n=367). The meta-analysis showed a trend to the benefit of TMP/SMZ use, but it did not reach a five per cent level of significance (OR 0.65, 95% CI 0.36-1.17). No heterogeneity was detected in this analysis (chi square for heterogeneity p=0.72, I²=0%). From 4 trials using quinolones data could be extracted.
Figure 2: Meta-analysis comparing SDD to no prophylaxis in which the outcome bacteremia is presented. 12 trials could be pooled. The first 7 trials compared TMP/SMZ to no SDD and the last 5 trials compared quinolones to no SDD. The Odds ratio (OR) is presented with a 95% confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>SDD n/N</th>
<th>no prophylaxis n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 TMP/SMZ trials</td>
<td>1/14</td>
<td>2/15</td>
<td>1.74</td>
<td>0.50</td>
<td>[0.04, 6.22]</td>
</tr>
<tr>
<td>Weiser 1981</td>
<td>3/24</td>
<td>7/23</td>
<td>6.05</td>
<td>0.33</td>
<td>[0.07, 1.46]</td>
</tr>
<tr>
<td>Gualtieri 1983</td>
<td>3/77</td>
<td>3/73</td>
<td>2.86</td>
<td>0.95</td>
<td>[0.18, 4.84]</td>
</tr>
<tr>
<td>Pizzo 1983</td>
<td>22/177</td>
<td>32/165</td>
<td>28.07</td>
<td>0.59</td>
<td>[0.33, 1.06]</td>
</tr>
<tr>
<td>EORTC 1984</td>
<td>2/20</td>
<td>6/23</td>
<td>4.86</td>
<td>0.31</td>
<td>[0.06, 1.78]</td>
</tr>
<tr>
<td>Henry 1984</td>
<td>1/43</td>
<td>7/48</td>
<td>6.25</td>
<td>0.14</td>
<td>[0.02, 3.18]</td>
</tr>
<tr>
<td>Kovatch 1985</td>
<td>6/22</td>
<td>7/20</td>
<td>5.16</td>
<td>0.70</td>
<td>[0.19, 2.59]</td>
</tr>
<tr>
<td>Ward 1993</td>
<td>377</td>
<td>367</td>
<td>55.00</td>
<td>0.51</td>
<td>[0.33, 0.79]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events: 38 (SDD), 64 (no prophylaxis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 3.05, df = 6 (P = 0.80), I² = 0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 2.99 (P = 0.003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 02 quinolone trials   |         |                     |                   |          |                   |
| Karp 1986             | 16/35   | 20/33               | 10.82             | 0.55     | [0.21, 1.44]      |
| Lew 1991              | 0/11    | 6/15                | 5.17              | 0.06     | [0.00, 1.28]      |
| Talbot 1993           | 10/62   | 22/57               | 18.61             | 0.31     | [0.13, 0.72]      |
| Carson 1997           | 6/45    | 6/45                | 5.03              | 1.00     | [0.30, 3.37]      |
| Lee 2002              | 2/46    | 6/49                | 5.38              | 0.33     | [0.06, 1.70]      |
| Subtotal (95% CI)     | 199     | 199                 | 45.00             | 0.42     | [0.25, 0.70]      |
| Total events: 34 (SDD), 60 (no prophylaxis) |
| Test for heterogeneity: Chi² = 4.39, df = 4 (P = 0.36), I² = 8.8% |
| Test for overall effect: Z = 3.35 (P = 0.0008) |
| Total (95% CI)        | 576     | 566                 | 100.00            | 0.47     | [0.34, 0.65]      |
| Total events: 72 (SDD), 124 (no prophylaxis) |
| Test for heterogeneity: Chi² = 7.73, df = 11 (P = 0.74), I² = 0% |
| Test for overall effect: Z = 4.45 (P < 0.00001) |

There were 5 patients with a Gram-negative bacteremia in the quinolone group (n=153) and 30 patients in the control group (n=150). There was a significant benefit for the use of quinolones. The OR for quinolone based regimens was 0.14 (95% CI 0.06-0.36) and no heterogeneity was detected in this analysis (chi square for heterogeneity p=0.65, I²=0%, Figure 3).

Effect on Gram-positive bacteremia
Ten trials reported on data concerning Gram-positive bacteremia. A total of 1005 patients were included. There were 41 patients with Gram-positive bacteremia in the SDD group (n=508) and 52 patients had a Gram-positive bacteremia in the control group (n=497). There was no significant difference between the 2 groups (OR 0.74, 95% CI 0.48-1.14). There was no heterogeneity in this analysis (Chi squared test for heterogeneity p=0.60, I²=0%). From 6 trials using TMP/SMZ the data could be extracted. There were 14 patients with a Gram-positive bacteremia in the TMP/SMZ group (n=355) and 28 patients in the control group (n=347).
Efficacy of selective decontamination of the digestive tract (SDD) in oncology patients

Figure 3 Meta-analysis comparing SDD to no prophylaxis in which the outcome Gram-negative bacteremia is presented. 11 trials could be pooled. The first 7 trials compared TMP/SMZ to no SDD and the last 4 trials compared quinolones to no SDD. The Odds ratio (OR) is presented with a 95% confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>SDD n/N</th>
<th>no Prophylaxis n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 TMP/SMZ trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weiner 1981</td>
<td>1/14</td>
<td>1/15</td>
<td>1.56</td>
<td>1.08</td>
<td>[0.06, 19.05]</td>
</tr>
<tr>
<td>Gualtieri 1983</td>
<td>2/24</td>
<td>6/23</td>
<td>9.76</td>
<td>0.26</td>
<td>[0.05, 1.44]</td>
</tr>
<tr>
<td>Pizzo 1983</td>
<td>1/77</td>
<td>1/73</td>
<td>1.76</td>
<td>0.95</td>
<td>[0.06, 15.43]</td>
</tr>
<tr>
<td>EORTC 1984</td>
<td>13/177</td>
<td>14/165</td>
<td>23.34</td>
<td>0.85</td>
<td>[0.39, 1.88]</td>
</tr>
<tr>
<td>Henry 1984</td>
<td>1/20</td>
<td>3/23</td>
<td>4.61</td>
<td>0.35</td>
<td>[0.03, 3.67]</td>
</tr>
<tr>
<td>Kovatch 1985</td>
<td>0/43</td>
<td>3/48</td>
<td>5.69</td>
<td>0.15</td>
<td>[0.01, 2.98]</td>
</tr>
<tr>
<td>Ward 1993</td>
<td>2/22</td>
<td>1/20</td>
<td>1.66</td>
<td>1.90</td>
<td>[0.16, 22.72]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>377</td>
<td>367</td>
<td>48.38</td>
<td>0.65</td>
<td>[0.36, 1.17]</td>
</tr>
<tr>
<td>Total events: 20 (SDD), 29 (no prophylaxis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi^2 = 3.68, df = 6 (P = 0.72), I^2 = 0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 1.44 (P = 0.15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 02 quinolone trials   |         |                    |                   |          |                   |
| Karp 1986             | 4/35    | 11/33              | 17.43             | 0.26     | [0.07, 0.92]      |
| Lew 1991              | 0/11    | 3/15               | 5.00              | 0.16     | [0.01, 3.34]      |
| Talbot 1993           | 1/62    | 13/57              | 23.17             | 0.06     | [0.01, 0.44]      |
| Carlson 1997          | 0/45    | 3/45               | 6.02              | 0.13     | [0.01, 2.66]      |
| Subtotal (95% CI)     | 153     | 150                | 51.62             | 0.14     | [0.06, 0.36]      |
| Total events: 5 (SDD), 30 (no prophylaxis) |
| Test for heterogeneity: Chi^2 = 1.64, df = 3 (P = 0.65), I^2 = 0% |
| Test for overall effect: Z = 4.07 (P < 0.0001) |

Total (95% CI): 530 517 100.00 0.39 [0.24, 0.63]

| Total events: 25 (SDD), 59 (no prophylaxis) |
| Test for heterogeneity: Chi^2 = 11.56, df = 10 (P = 0.32), I^2 = 13.5% |
| Test for overall effect: Z = 3.89 (P = 0.0001) |

There was a benefit shown to reduce Gram-positive bacteremia with the use of TMP/SMZ (OR 0.47, 95% CI 0.25-0.91). There was no heterogeneity in this analysis (Chi squared test for heterogeneity p=0.96, I^2 =0%). From 4 trials using quinolones the data could be extracted. There were 27 patients with a Gram-positive bacteremia in the quinolone group (n=153) and 24 patients in the control group (n=150). There was no significant difference found (OR 1.11, 95% CI 0.6-2.05). There was no heterogeneity in this analysis (Chi squared test for heterogeneity p=0.44, I^2 =0%, Figure 4).

Effect on fungemia

Eleven trials reported on the outcome fungemia. Of these 11 trials 4 used antifungal prophylaxis in addition to the SDD regimen given. There were 14 patients with fungemia in the SDD group (n=531) and 9 patients with fungemia in the control group (n=521). The difference between both groups was not significant (OR 1.32, 95% CI 0.60-2.91). This analysis did not show heterogeneity (Chi squared test for heterogeneity p=0.94, I^2 =0%).
**Figure 4**: Meta-analysis comparing SDD to no prophylaxis in which the outcome Gram-positive bacteremia is presented. 10 trials were pooled. 6 trials compared TMP/SMZ to no SDD, and 4 trials compared quinolones to no SDD. The Odds ratio (OR) is presented with a 95% confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>SDD n/N</th>
<th>no prophylaxis n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 TMP/SMZ trials</td>
<td>0/14</td>
<td>1/15</td>
<td>2.98</td>
<td>0.33</td>
<td>[0.01, 8.88]</td>
</tr>
<tr>
<td></td>
<td>1/24</td>
<td>1/23</td>
<td>2.08</td>
<td>0.96</td>
<td>[0.06, 16.25]</td>
</tr>
<tr>
<td></td>
<td>2/77</td>
<td>2/73</td>
<td>4.25</td>
<td>0.95</td>
<td>[0.13, 6.90]</td>
</tr>
<tr>
<td></td>
<td>9/177</td>
<td>18/165</td>
<td>37.54</td>
<td>0.44</td>
<td>[0.19, 1.00]</td>
</tr>
<tr>
<td></td>
<td>1/20</td>
<td>2/23</td>
<td>3.75</td>
<td>0.55</td>
<td>[0.05, 6.59]</td>
</tr>
<tr>
<td></td>
<td>1/43</td>
<td>4/48</td>
<td>7.84</td>
<td>0.26</td>
<td>[0.03, 2.44]</td>
</tr>
<tr>
<td></td>
<td>355</td>
<td>347</td>
<td>58.43</td>
<td>0.47</td>
<td>[0.25, 0.91]</td>
</tr>
<tr>
<td>Total events: 14 (SDD), 28 (no prophylaxis) Test for heterogeneity: Chi² = 1.07, df = 5 (P = 0.96), I² = 0% Test for overall effect: Z = 2.26 (P = 0.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 02 quinolone trials   | 12/35   | 9/33             | 12.92             | 1.39     | [0.49, 3.92]      |
|                       | 0/11    | 3/15             | 6.10              | 0.16     | [0.01, 3.34]      |
|                       | 9/62    | 9/57             | 17.02             | 0.91     | [0.33, 2.47]      |
|                       | 6/45    | 3/45             | 5.52              | 2.15     | [0.50, 9.21]      |
|                       | 153     | 150              | 41.57             | 1.11     | [0.60, 2.05]      |
| Total events: 27 (SDD), 24 (no prophylaxis) Test for heterogeneity: Chi² = 2.72, df = 3 (P = 0.44), I² = 0% Test for overall effect: Z = 0.34 (P = 0.73) |

Total (95% CI) 508 497 100.00 0.74 [0.48, 1.14]

**Effect on reducing infection related mortality**

Out of 13 trials data could be extracted on infection-related mortality. Four trials did not report on mortality data. A total of 1116 patients were included in this analysis. There were 23 patients who died due to an infectious cause in the SDD group (n=564) and there were 39 patients who died because of an infectious cause in the control group (n=452). A significant benefit was shown in the use of SDD (Odds ratio 0.56 95% CI 0.34-0.96). In this analysis no heterogeneity was found (chi square for heterogeneity p=0.54, I² =0%). In both groups an equal number of fungal infections were found. Excluding fungal causes of death, as SDD does not cover fungal organisms, we found 16 patients who died due to a bacteremia (n=478) in the SDD group and 32 patients who died due to a bacteremia in the control group (n=478). Thus, there was also a decrease of infection-related mortality due to bacterial causes (Odds ratio 0.49, 95% CI 0.27-0.88). No heterogeneity in this analysis was found (chi square for heterogeneity p=0.62, I² =0%, Figure 5).
Figure 5: Meta-analysis comparing SDD to no prophylaxis in which the outcome infection-related mortality due to bacterial causes is presented. 13 trials were pooled. 7 trials compared TMP/SMZ to no SDD, and 6 trials compared quinolones to no SDD. The Odds ratio (OR) is presented with a 95% confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 TMP/SMZ trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kramer 1982</td>
<td>3/35</td>
<td>1/31</td>
<td>2.96</td>
<td>2.81</td>
<td>[0.28, 28.54]</td>
</tr>
<tr>
<td>Gualtieri 1983</td>
<td>1/24</td>
<td>2/23</td>
<td>5.98</td>
<td>0.46</td>
<td>[0.04, 5.41]</td>
</tr>
<tr>
<td>Kauffmann 1983</td>
<td>0/29</td>
<td>6/26</td>
<td>20.57</td>
<td>0.05</td>
<td>[0.00, 1.00]</td>
</tr>
<tr>
<td>de Jongh 1983</td>
<td>2/32</td>
<td>4/29</td>
<td>12.03</td>
<td>0.42</td>
<td>[0.07, 2.47]</td>
</tr>
<tr>
<td>Henry 1984</td>
<td>3/20</td>
<td>4/23</td>
<td>9.67</td>
<td>0.84</td>
<td>[0.16, 4.29]</td>
</tr>
<tr>
<td>Kovatch 1985</td>
<td>0/43</td>
<td>2/48</td>
<td>7.15</td>
<td>0.21</td>
<td>[0.01, 4.58]</td>
</tr>
<tr>
<td>Ward 1993</td>
<td>3/22</td>
<td>3/20</td>
<td>8.30</td>
<td>0.89</td>
<td>[0.16, 5.04]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>205</td>
<td>200</td>
<td></td>
<td>66.65</td>
<td>0.51 [0.25, 1.05]</td>
</tr>
<tr>
<td>Total events: 12 (Treatment), 22 (Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Test for heterogeneity: Chi$^2 = 5.47$, df = 6 (P = 0.48), $I^2 = 0$
Test for overall effect: Z = 1.83 (P = 0.07)

| 02 quinolone trials  |               |             |                   |          |                  |
| Karp 1986             | 2/35          | 2/33        | 5.93              | 0.94     | [0.12, 7.08]     |
| Lew 1991              | 0/11          | 0/15        | Not estimable     |          |                  |
| Talbot 1993           | 0/62          | 1/57        | 4.74              | 0.30     | [0.01, 7.55]     |
| Carlson 1997          | 0/45          | 0/45        | Not estimable     |          |                  |
| Tjan-Heijnen 2001     | 0/83          | 5/80        | 17.02             | 0.08     | [0.00, 1.51]     |
| Lee 2002              | 2/46          | 2/49        | 5.66              | 1.07     | [0.14, 7.91]     |
| Subtotal (95% CI)     | 282           | 279         |                   | 33.35    | 0.43 [0.15, 1.27]|
| Total events: 4 (Treatment), 10 (Control) |
Test for heterogeneity: Chi$^2 = 2.64$, df = 3 (P = 0.45), $I^2 = 0$
Test for overall effect: Z = 1.53 (P = 0.13)

Quinolone versus TMP/SMZ

In the four trials comparing quinolones to TMP/SMZ a total of 525 patients were included (Table 2). In the quinolone group 5 patients had a Gram-negative bacteremia (n=249) and in the TMP/SMZ group (n=242) 14 patients had a Gram-negative bacteremia. This showed a benefit in the use of quinolones (OR 0.37, 95% CI 0.14-0.99). However, there was heterogeneity between the studies (chi square for heterogeneity $p=0.26$, $I^2=25.3$%) and this result should be interpreted with caution.

There were 37 patients with Gram-positive bacteremia in the quinolone group (n=249) and 14 patients in the TMP/SMZ group (n=242). There was a significant benefit found in the use of TMP/SMZ over quinolones. The OR was 2.39 (95%CI 1.37-4.16). There was no heterogeneity between studies. If we assessed infection-related mortality there were 13 patients who died in the group using quinolones (n=249), and 5 patients died in the group using TMP/SMZ (n=242). The OR was 2.70 (95% CI 0.94-7.8), which means that TMP/SMZ based regimens may be more
effective than quinolone based regimens in decreasing infection related mortality; yet, this did not reach a five per cent level of significance.

**Subgroup analyses**

1. Conventional chemotherapy vs. bone marrow transplant

Of 12 SDD trials the data could be abstracted to compare patients receiving conventional chemotherapy (9 trials) and patients who underwent a bone-marrow transplant (BMT) procedure (3 trials). In the 9 conventional chemotherapy trials a total of 712 patients were included. If we analysed the outcome bacteremia, there were 34 patients with bacteremia in the SDD group (n=353) and 69 patients in the control group (n=359). A benefit was shown in favor of the use of SDD in patients receiving conventional chemotherapy. The odds ratio was 0.41 (95% CI: 0.26-0.64). There was no heterogeneity in this analysis (chi square for heterogeneity $p=0.58$, $I^2 =0\%$) There were 3 trials only including BMT patients with a total of 436 patients. If we analysed the same outcome bacteremia there were 38 patients with bacteremia in the SDD group (n=223) and 58 patients in the control group (n=213). A benefit was shown in favor of the use of SDD in patients receiving a BMT procedure. The OR was 0.52 (95% CI: 0.32-0.84). No heterogeneity was found in this analysis (chi square for heterogeneity $p=0.35$, $I^2 =3.7\%$). In this subgroup analysis both groups benefit equally from the use of SDD.

**Pediatric trials**

There were three included trials with pediatric patients (see Table 3). Three trials compared TMP/SMZ to placebo, but from only 2 trials the pediatric data could be included, one trial combined adult and pediatric results. Pooling of these data is not possible. The EORTC trial only provided information on the outcome (in pediatric patients. In the TMP/SMZ group (n=33) there were 2 patients with bacteremia and in the control group also 2 patients (n=32). The Kovatch trial provided information on both bacteremia and Gram-negative bacteremia. In the TMP/SMZ group (n=43) there were no patients with Gram-negative bacteremia, and in the control group there were 3 patients (n=48). The last trial from Pizzo et al. did not separate the pediatric data from the adult data.

**Discussion**

This systematic review showed a significant reduction in both Gram-negative bacteremia and mortality due to bacterial infections, without an increase in fungal septicaemia's in the SDD group. Earlier systematic reviews showed a significant reduction of Gram-negative bacteremia with quinolone-based regimens but failed to show a reduction in infection-related mortality. There are several explanations for this difference. First, in our systematic review we included trials both on TMP/SMZ as on quinolones. Cruciani and Engels only focussed on quinolone
Efficacy of selective decontamination of the digestive tract (SDD) in oncology patients

regimens. Second, in our analyses we compared TMP/SMZ or quinolone based regimens with no treatment or placebo as the previous reviews also included trials with other antibiotics in the comparison groups. Third, we excluded trials on the intravenous use of SDD prophylaxis. Fourth, we focussed on trials in which patients started SDD prophylaxis before the onset of neutropenia (which is essential for the appropriate use of SDD) and in which patients had no infections at the time of inclusion. Fifth we only abstracted data from trials where the number of patients with bacteremia (Gram-negative or Gram-positive) could be compared to the total number of patients per treatment group.

The incidence of bacteremia is reduced by the use of both TMP/SMZ and quinolone prophylaxis. Because of the high risk of mortality due to Gram-negative bacteria one aim of SDD treatment is to reduce Gram-negative bacteremia. Both TMP/SMZ and quinolone prophylaxis decrease the risk of Gram-negative bacteremia. This systematic review shows that quinolone based SDD regimens are more effective in reducing Gram-negative bacteremia's than TMP/SMZ based regimens. Concerning the Gram-positive bacteremia our systematic review shows that with TMP/SMZ based regimens the incidence of Gram-positive bacteremias is reduced. This is not evident for quinolone based SDD regimens. It is well known that quinolones offer inadequate coverage for Gram-positive infections. Addition of antibiotics covering Gram-positive microorganisms, could address this problem. Cruciani et al summarised all RCT's performed on the addition of Gram-positive prophylaxis to fluoroquinolones in a systematic review and concluded that there is lack of clear cut benefit on morbidity and mortality. This treatment strategy, however, should be valuable in subgroups of patients at high risk of streptococcal infections. This has yet to be shown.

Although there is an increase over time of Gram-positive organisms 60-70% , the mortality due to infections is much higher for Gram-negative bacteremia, than for Gram-positive bacteremia. Celkan et al describe the infection related mortality in oncology patients as 8% due to Gram-positive septicaemia’s and 20% due to Gram-negative septicaemia’s. Therefore it is essential to focus on elimination of Gram-negative organisms.

One issue of great concern in the oncology patient is the occurrence of resistant isolates. Strains that have shown to develop quinolone resistance are E. Coli and coagulas e negative staphylococcus. The percentage of emerging resistance differs from more than 25% reported in the EORTC trials to less than 10% in the Memorial Sloan Kettering Center. It is likely that the increasing resistance to fluoroquinolones among isolates of cancer patients reflects the pressure put on the endogenous flora, rather than dissemination of fluoroquinolone resistant strains in the general population. Data on resistance in our the trials included in this review were difficult to interpret, and not all trials reported on resistance, therefore no conclusion can be drawn on development of resistant organisms from these data.

The fear of developing resistant organisms raises the question if we can stratify the use of SDD to certain high-risk groups of patients. Therefore a sub analysis was performed. A benefit was
shown for the use of SDD in both patients who undergo conventional chemotherapy and patients who undergo a BMT procedure. The explanation that both groups benefit might be due to the intensity of conventional chemotherapy which is comparable to a BMT procedure. We know that quinolones provide poor coverage for streptococci and coagulase-negative staphylococci, therefore there is a higher risk of colonization of these organisms and increased chance for infection. In our meta-analysis we found that the incidence of Gram-positive bacteremia did not increase with the use of SDD (OR 0.73, 95% CI 0.47-1.11). A sub analysis performed in pediatric trials included the only three trials. Infection-risk was much lower in these trials than in adult trials which is in keeping with the literature. The data do not allow us to draw conclusions on pediatric patients only. It is, however, known that the risks for infection in the patient with cancer are similar for both adults and children. We believe that the data shown on the use of SDD in adults are also relevant to the pediatric oncology patients. In conclusion, this systematic review shows that TMP/SMZ or quinolone based SDD regimen started before the onset of neutropenia reduces both Gram-negative bacteremia and infection related mortality in neutropenic oncology patients. The risk of developing resistant strains could not be evaluated but the use of TMP/SMZ or quinolone prophylaxis did not increase the risk of Gram-positive bacteremia or fungemia.

Implications for clinical practice
We believe that TMP/SMZ or quinolone prophylaxis is beneficial to cancer patients with a high baseline risk for infections, such as patients with hematologic-hematological malignancies, autologous and allogenic bone-marrow transplant patients and solid tumor patients who have an expected neutropenia of at least 7 days. We therefore advise to give SDD to oncology patients with an expected base-line infection risk of more than 10%. In such patients, if the infection risk is e.g. 20% one would have to treat 5 patients to prevent one Gram-negative bacteremia with a quinolone based regimen (NNT=5). To prevent one infection related death with a quinolone or TMP/SMZ based regimen in patients at a baseline risk of infection-related mortality of 2% one would have to treat 100 patients (NNT=100). The regimen used will depend on the best available data on development of resistance, and strict monitoring to detect resistant strains will be an absolute necessity.

Acknowledgements:
We would like to thank Dr. P.J.M. Bakker, medical oncologist, Academic Medical Center, Amsterdam for critically reviewing this article.
Reference List


## Appendix 1

<table>
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Chapter 7

A child with infant ALL and severe varicella-zoster pneumonia

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A little boy, presented at the age of 6 months with infant leukemia. He was started on the acute lymphoblastic leukemia protocol for children <1 year and had a good response. After the induction period he was in complete remission. After one year of intensive chemotherapy he started his maintenance therapy, consisting of dexamethasone 6mg/m²/day x 14 days with vincristine 1.5 mg/m² on day 1 and day 7 intravenously followed by 5 weeks oral 6-mercaptopurine 50 mg/m²/daily and methotrexate 20 mg/m² weekly, after this the cycle repeated.

He was on maintenance therapy for 3 months when he developed varicella zoster vesicles all over the body, but otherwise was not ill at that stage. It was known that this boy was IgG negative for varicella zoster. The contact of this varicella infection was not known, therefore he did not receive the passive immunisation within 72 hours of zoster-immunoglobulin. On developing the vesicles he was started on high dose oral aciclovir and was admitted to hospital. Initially he was an otherwise well toddler except for the extensive vesicles. He did not improve and developed severe abdominal pain 10 days after the first vesicles had appeared. He was not vomiting, no diarrhea, and the pain did not seem related to the food intake. He was however not well at this stage, the vesicles were turning hemorrhagic (fig 1), and he was agitated, he did not want to walk anymore. He was started on i.v aciclovir and because of the possibility of secondary infection Augmentin® i.v. was started.

The following day this little boy deteriorated and was distressed. His respiration rate was 50/ min and on auscultation bilateral rhonchi were heard. His eyes were edematous and he was very irritated. He deteriorated rapidly that day and had to be transferred to the intensive care unit to be mechanically ventilated. At this stage he had an extreme high temperature and was neutropenic, therefore he was started on a cephalosporin and gentamycin. His chest X-ray showed bilateral infiltrative changes which progressed from fairly visible changes to a total white out (fig 2). The viral load of varicella measured with a PCR in blood was extremely high 1600.000c/ml, also the PCR from the broncho-alveolar lavage was positive for VZV, therefore it was clear that the complication this boy had was a varicella-pneumonia.
While on conventional ventilation he deteriorated despite pressures of 32/20 mm Hg, and FiO2 of 100%. Therefore he was started on high frequency oscillation ventilation. He reacted well to this form of ventilation, and could be extubated after 8 days. The viral load very slowly decreased. Slowly he improved and i.v. therapy was continued for 4 weeks, then his viral load showed 2500 c/ml and at this stage his therapy was switched from intravenous to oral therapy famciclovir.

**Figure 2:** Chest X ray presenting the varicella zoster pneumonia at time of HFO ventilation.

**Figure 3:** Viral load longitudinal data, showing that T cell response recovered after nearly one year and at that stage the viral load was 0.
which was continued another 9 months. Then he did show an adequate T-cell immune-response, and it was decided to stop the oral treatment (Fig 3). He has done very well since. He finished his chemotherapy and has been in complete remission for over 2 years after stop of the chemotherapy.

**Varicella pneumonia**

This represents a severe complication of varicella, mostly seen in the immuno-compromised patients and in adults. This severe complication carries a mortality of 10-30% (1). However, when respiratory failure occurs and mechanical ventilation is necessary, mortality is as high as 50% (2,3). Although we know that aciclovir has limited efficacy it remains the first-line therapy (1). Varicella pneumonia causes an interstitial pneumonitis with impairment of pulmonary gas-exchange. The pneumonitis is probably due to host response rather than virally mediated tissue injury.

Corticosteroids may modify the inflammatory response. This results in a decrease in the release of macrophage-derived pro-inflammatory cytokines like interleukin-1 and Tumor necrosis factor-α (4, 5) and a decrease in production of membrane-derived products like leukotrienes and prostaglandins, leading to less edema and improved vascular permeability. Therefore it is recommended to start steroids in addition to anti-viral therapy and other supportive care measurements. This little boy did not cope on conventional chemotherapy, fortunately he responded to high frequency oscillation ventilation. Another form of ventilation these patients have been responding to fairly well is ECMO ventilation (extracorporeal membrane oxygenation). Early recognition of pulmonary failure and rapid institution of ECMO are critical in the successful management of this complication of varicella zoster infection. ECMO as such does little to reverse the course of the underlying disease. It’s role is one of support, during which time the lungs are rested and allowed to recover(6, 7).

**References**

Chapter 7

Varicella vaccination in an early phase of chemotherapy in pediatric oncology patients

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* Authors contributed equally to this work

Study in progress
Abstract

The incidence of morbidity and mortality from primary varicella-zoster virus (VZV) infection is increased in immunocompromised children. Vaccination of VZV-seronegative cancer patients with live attenuated varicella vaccine has proven to be safe and effective. However, all vaccination programs performed so far in this group of patients required the interruption of chemotherapy. This is the first cohort of pediatric oncology patients who received the live attenuated varicella zoster vaccine in an early phase of their treatment without interrupting chemotherapy. Eleven patients with either a hematological malignancy (n=8) or a solid tumor (n=3) were vaccinated with VZV-vaccine during chemotherapy. Seroconversion occurred in 8 of the 11 patients (72.7%) after one vaccination. The only adverse effects consisted of a mild rash (10-50 lesions n=2), a moderate vesico-papular rash (50-200 lesions n=1), mild gastro-intestinal symptoms (n=1), and mild respiratory complaints (n=1). VZV-specific CD4+ T cells could not be detected by intracellular IFN-g staining during follow-up. However, adaptive immunity was induced since seroconversion was observed in 72.7% of the patients. This study demonstrates that it is feasible to administer VZV-vaccine in an early stage of chemotherapy without interruption of chemotherapy.
Introduction

Varicella (chickenpox) is a highly infectious, usually self-limiting disease caused by the α-herpesvirus family member varicella-zoster virus (VZV). Children generally develop mild disease, manifested by fever, a vesicular rash and mild constitutional symptoms. However, in immunocompromised children such as children with a malignancy, the incidence of complications and mortality due to VZV infection is highly increased. The complication-rate in these children is approximately 30% and the mortality-rate approaches 20% in untreated cases [10]. Antiviral therapy has improved the outcome considerably but the overall mortality rate in the immunocompromised patient remains 7% [10,15]. The most common complication is acute bacterial skin-infection, caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. The incidence of bacterial superinfections is increased in children under 5 years of age, and this complication may lead to a streptococcal toxic shock syndrome [23]. Furthermore, neurologic complications are more often observed in the immunocompromised. Postinfectious cerebellar ataxia usually resolves without further complications, whereas meningoencephalitis has a much less favorable outcome. The mortality rate from this latter complication ranges from 5-25% and neurologic sequelae are seen in 20% of patients [4]. Less frequent complications include pneumonia, visceral complications (including hepatitis and severe gastro-intestinal symptoms) and hematological complications (e.g. thrombocytopenia, pancytopenia) and the development of hemorrhagic varicella.

As awareness of the morbidity and mortality due to VZV infection became established, the interest in the live attenuated vaccine was increased. The vaccine was developed in Japan in the early 1970’s from a viral strain (Oka-strain) isolated from an infected patient and attenuated by passage through cell cultures [19]. The vaccine 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 US are including varicella vaccination in their routine schedule. The vaccine induces long-term humoral and cellular immunity in children and adults [5,24]. Since the implementation of VZV vaccination, the US has seen a marked decline in the number of cases of varicella and a trend towards less hospitalizations due to chickenpox [18].

Since the incidence of complications of VZV infection is increased in immunocompromised patients as compared to otherwise healthy individuals, it would be of great benefit to vaccinate VZV-seronegative oncology patients. In the past, 575 children with leukemia in remission were immunized in the Varicella Vaccine Collaborative Study [14]. All children were in continuous remission for more than one year, and had more than 700/mm³ circulating lymphocytes. Chemotherapy was stopped 1 week before and 1 week after immunization in the majority of these children. Recommendations included withdrawal of steroids for 2 weeks after immunization. The varicella vaccine proved to be safe immunogenic and effective. The major adverse reaction was a varicelliform rash, which could be treated in most cases with oral aciclovir. Seroconversion to VZV occurred in 82% of vaccinees after 1 dose and in 95% after 2 doses. In
addition, the incidence of clinical reactivation (herpes zoster) in vaccinated children is lower than in unvaccinated leukemic children. Although it is believed that cell-mediated immunity is elicited less reliably in leukemic children after varicella vaccination than in their healthy counterparts [11,14]. The study indicated that administration of VZV vaccine under these conditions is extremely beneficial to leukemic patients.

Because of the great benefits for the patients, VZV vaccination should be extended to other oncology patients and preferably in an earlier phase of treatment. One study on vaccination of patients before the start of chemotherapy resulted in seroconversion in 10 out of 13 vaccinated children (77%) [8]. Mild side-effects were observed in 12.5% of the patients consisting of a varicelliform rash and fever. Since only mild side-effects were observed in the patient group, it seemed safe to administer the vaccine.

In both of these studies, chemotherapeutic treatment was delayed in order to safely vaccinate these children. Although it is clear that the risk of developing complicated varicella upon VZV infection is decreased, the required delay of chemotherapeutic treatment of the malignancy in these children could have a negative effect on the clinical outcome. In Japan in the 80’s, the vaccine was administered without stopping chemotherapy but not respecting the criteria of stopping steroids 14 days before vaccination and one week after vaccination, having an adequate number of lymphocytes (>700/mm³), and not having bulky disease at the time of vaccination. This led to an unacceptably high incidence of complications and severity of rashes [1,12,17].

The aim of our study was to investigate the efficacy of VZV vaccination of seronegative pediatric oncology patients without interrupting chemotherapy and introducing the vaccine in an early phase of treatment, respecting the rule not to vaccinate during the phase in which steroids are given, or when there is a low number of circulating lymphocytes, or when active hematologic malignancy is present.

Materials & Methods

Patient selection

Newly diagnosed pediatric oncology patients who were seronegative for VZV were included in this study. Eligibility criteria included patients on chemotherapy, who had circulating lymphocyte counts of >700/mm³, had no bulky disease and were not septic at the time of vaccination. Before and after vaccination no steroids were given in the regimen for at least one week, the vaccination did not lead to interruption of chemotherapy but was given in a phase when no steroids were needed. The study was performed in a single pediatric oncology unit and approved by the local medical ethical committee. Written informed consents were obtained from the parents of the patients and from the patient if >12 years of age.
Varicella vaccination in an early phase of chemotherapy in pediatric oncology patients

Vaccination protocol
Varicella vaccine (Varilrix, GlaxoSmithKline UK, Uxbridge, UK) containing the live-attenuated VZV Oka-strain was administered subcutaneously to the eligible patients (day 0). The time point of vaccination varied among the different protocols and clinical situations. Clinical scores were registered both on basis of questionnaires, and physical examination on day 0, 7, 14, 21, 28 and 3 months after vaccination. Parameters recorded by the questionnaires included: fever (>39°C), headache, vomiting, diarrhea (defined as >6 loose stools a day), coughing, and pain, rash or induration at the injection-site. Parameters recorded on physical examination included: temperature, organomegaly (upon vaccination), skin lesions, and signs of infection. Standard laboratory tests were concomitantly performed, consisting of a full blood count, determination of levels of creatinin, LDH and liver enzymes (ASAT, ALAT). Furthermore, peripheral blood samples were drawn from the patients at each visit, and throat-swabs were taken (twice a week) to determine the development of an immunological response to the virus, as well as contagiousness of the vaccinated patients. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples using standard density gradient centrifugation techniques by use of Lymphoprep (Nycomed, Pharma, Oslo, Norway). PBMCs were cryopreserved until use and thawed according to standard procedures.

Laboratory parameters for VZV infection
Viral culture of vesicle fluids or scrapings was done by cocultivation with human embryo lung fibroblast cells and microscopic examination of VZV-specific cytopathological effects. Confirmation was done by immunofluorescent staining of cells with murine monoclonal anti-VZV immunoglobulin M (IgM) (BioWhittaker Inc., Walkersville, Md.). VZV IgM and IgG titers were determined in plasma as described before by use of the miniVidas (Biomerieux, Marcy l’Etoile, France) [9]. Results are expressed as arbitrary units/ml serum.
Quantitative PCR to determine VZV viral load was performed in plasma samples as well as in throat swabs described before [9]. The electrochemiluminescence (ECL) signal was measured by an M-8 analyzer (IGEN, Oxford, UK).

Determination of VZV-specific CD4+ T cells by intracellular cytokine staining
VZV-specific CD4+ T-cell frequencies were determined as described previously for cytomegalovirus (CMV)-specific cells [21]. In short, PBMCs were resuspended in RPMI, containing 10% fetal calf serum (FCS) and antibiotics, and stimulated for 6 hours with VZV-antigen (20 ml/ml; Microbix Biosystems, Toronto, Canada), the final 5 hours in presence of brefeldin-A (10 mg/ml). VZV antigen is a lysate of VZV-infected cells, containing a broad range of virus-derived peptides. PBMCs were costimulated by CD28 (2 µg/ml; CLB 15E8) and CD49d (1 µg/ml; BD Biosciences (BD), San Jose, CA). Cells were permeabilised using the BD-FACS intracellular cytokine staining kit (according to the manufacturer’s instructions) and stained for IFN-γ-FITC, CD4-PerCP-Cy5.5
(all BD) and CD69-APC (Caltag Laboratories, Burlingame, CA). The CD4+CD69+IFN-γ+ T cells were designated antigen-specific CD4+ T cells. Negative controls consisted of stimulation with medium and positive controls of stimulation with Staphylococcus Aureus enterotoxin B (SEB; Sigma, St Louis, MO).

**Immunofluorescent staining and flowcytometry**

Whole blood samples were used to determine the absolute numbers and percentages of CD4+ and CD8+ T cells, B cells and NK cells by use of the Multi Test IMK kit (BD). 20 ml of multiset antibody mix, either CD3/CD8/CD45/CD4 or CD3/CD16/CD56/CD45/CD19 were added to 50 ml blood in two different TruCount tubes (BD). Samples were vortexed thoroughly and incubated for 30 min. at room temperature (RT), protected from light. Next, 10x diluted lysis buffer was added to the samples. After vortexing, samples were incubated for 15 min at RT, protected from light. Absolute numbers and percentages of lymphocyte cell populations were determined by the Multiset program (BD) on a FACSCalibur (BD). Controls were included in each experiment.

**Statistics**

The main findings of the study are described descriptively. Where applicable, differences between means were tested by Student t test.

**Results**

**Patient characteristics**

Eleven VZV seronegative pediatric oncology patients were included in this study from February 2002 until November 2003. The time between start of chemotherapy and vaccination was 9.6 weeks ± 2.1 weeks. Eight of these patients had a hematological malignancy (NHR-ALL; n=4, HR-ALL; n=4), whereas the other three had a solid tumor (Table 1). The age at vaccination (5.5 ±

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<tr>
<th>Patient</th>
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1 t=0: time point of varicella vaccination, NHR-ALL: non-high-risk acute lymphoblastic leukemia, HR-ALL: high-risk acute lymphoblastic leukemia
4.6 vs. 4.2 ± 1.9 years, resp.) did not differ significantly between the two groups of malignancies at the time of varicella vaccination (Table 1). Of note, chemotherapy was not stopped or delayed because of the varicella vaccination in any of these patients.

**Laboratory results (Table 2)**

On the day of vaccination, the mean Hb was 6.8 mmol/L (range 5.5-7.6 mmol/L), which remained stable during follow-up. The mean white blood cell count (wcc) was 5.8 x10⁹/L (range 2.2-10.2 x10⁹/L) at day 0. The mean neutrophil count was 2422 cells/L (range 770-4650 cells/L). The mean lymphocyte-count was 2674 cells/L (range 774-6830 cells/L). Raised liver enzymes were observed in 4 patients, which increased further in the next two weeks after vaccination (data not shown). These patients however were patients with leukemia receiving high dose intravenous methotrexate, which could well explain the disturbance in liver enzymes. Liver enzymes normalized in all patients within three months after vaccination.

No significant differences in the numbers of circulating white blood cells, lymphocytes or neutrophils were detected between the patients with a hematological malignancy and those with a solid tumor (Fig. 1).

![Figure 1](image.png) Absolute numbers of white blood cells, lymphocytes and neutrophils on day of vaccination. No statistical significant differences in the absolute numbers of white blood cells, neutrophils and lymphocytes could be detected on day 0 between the patients with a hematological malignancy and those with a solid tumor.
Table 2 Laboratory test results on day 0 of vaccination

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb (mmol/L)</th>
<th>Neutrophils (cells/µL)</th>
<th>Lymphocytes (cells/µL)</th>
<th>Thrombocytes (cells/µL)</th>
<th>Liver enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.7</td>
<td>3500</td>
<td>1500</td>
<td>290</td>
<td>4x normal*</td>
</tr>
<tr>
<td>2</td>
<td>7.6</td>
<td>2500</td>
<td>6830</td>
<td>243</td>
<td>normal</td>
</tr>
<tr>
<td>3</td>
<td>6.8</td>
<td>3000</td>
<td>2580</td>
<td>350</td>
<td>5x normal*</td>
</tr>
<tr>
<td>4</td>
<td>6.6</td>
<td>858</td>
<td>5600</td>
<td>360</td>
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</tr>
<tr>
<td>5</td>
<td>6.2</td>
<td>1800</td>
<td>2200</td>
<td>326</td>
<td>normal</td>
</tr>
<tr>
<td>6</td>
<td>6.5</td>
<td>4650</td>
<td>1280</td>
<td>440</td>
<td>2x normal*</td>
</tr>
<tr>
<td>7</td>
<td>6.9</td>
<td>770</td>
<td>1180</td>
<td>492</td>
<td>2x normal*</td>
</tr>
<tr>
<td>8</td>
<td>7.1</td>
<td>3354</td>
<td>774</td>
<td>335</td>
<td>normal</td>
</tr>
<tr>
<td>9</td>
<td>7.3</td>
<td>2180</td>
<td>798</td>
<td>235</td>
<td>normal</td>
</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>3082</td>
<td>780</td>
<td>286</td>
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</tr>
<tr>
<td>11</td>
<td>7.1</td>
<td>1450</td>
<td>2700</td>
<td>222</td>
<td>normal</td>
</tr>
</tbody>
</table>

*patients with leukemia received high-dose Methotrexate i.v.

Low incidence of adverse effects

In five of the patients (45.4%), mild adverse effects were observed (Table 3). In the first week after vaccination, patient 1 had a painful injection site and developed a rash. Vesicles were observed on the skin starting from the first week (10-50), which increased in the second week (50-200). Treatment consisted of intravenous aciclovir, later followed by oral famciclovir. Pt. 3 developed 10-50 lesions on her skin during the second week after vaccination, which was treated by oral famciclovir. Pt. 5 developed a rash at the injection site in the second week after vaccination. An exanthem was observed on his skin starting from the first week after vaccination, which reduced to 10-50 lesions within the second week. Treatment consisted of oral famciclovir. Two of these patients (pt. 1 and 5) developed a fever within the first week after vaccination, which continued for 2 weeks. Mild gastro-intestinal symptoms were observed in pt. 11 between the first and the second week after vaccination. Pt. 9 developed mild respiratory complaints, between the second and fourth week after vaccination. Other adverse effects were not observed in our cohort. According to the Vazquez score [20]

Table 3 Adverse effects

<table>
<thead>
<tr>
<th>Patient</th>
<th>injection site</th>
<th>rash(# lesions)</th>
<th>treatment</th>
<th>Fever</th>
<th>Vazquez score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pain day 7</td>
<td>50-200</td>
<td>famciclovir and iv aciclovir</td>
<td>day 7-10</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>pain day 7</td>
<td>10-50</td>
<td>famciclovir</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>pain day 7</td>
<td>10-50</td>
<td>famciclovir</td>
<td>day 7-14</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1*</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1*</td>
</tr>
</tbody>
</table>

* pt. 9 had mild respiratory complaints, and pt. 11 had mild gastro-intestinal complaints, scored as 1 according to the Vazquez score [20]
to the clinical score of Vazquez et al [20, appendix 1], all patients scored <7 points (max 2 points) and were considered to have mild disease (Table 3).

**Efficacy of vaccination**

VZV-specific IgG could be detected upon vaccination in 8 of the 11 patients (72.7%) after the first vaccination (Table 4). Seroconversion occurred within 6 weeks after varicella vaccination in 6 of the seroconverting patients (75%). In the other 2 patients, seroconversion occurred within 3 months after vaccination (25%). Three of the patients did not seroconvert upon varicella vaccination. One of these patients was diagnosed as NHR-ALL, one as HR-ALL, and one had a nephroblastoma. Seroconversion did not correlate with the age at vaccination, the time between start of chemotherapy and vaccination, or with the number of circulating lymphocytes at the time of vaccination (Figure 2). The patients that did not seroconvert upon varicella vaccination are scheduled for booster vaccination.

Household contacts to wild-type VZV were documented in two of the patients. The first patient (pt. 10) showed no clinical signs of varicella upon contact and was therefore not treated. Seroconversion could not be observed in this patient after vaccination, neither after exposure to the wild-type virus. The second patient (pt. 3), who seroconverted within 6 weeks after varicella vaccination, developed a varicellaform rash (50-200 lesions) and high fever within 3 days after contact (6 months after vaccination). She had a Vazquez-score of 13 and appeared moderately ill. She recovered rapidly after start of iv aciclovir. She had a

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**Figure 2** Correlation of seroconversion to possible risk factors. Seroconversion did not correlate to the age at vaccination, the time between start of chemotherapy, nor to the number of circulating lymphocytes at the time of varicella vaccination.
relative low viral load (max. VZV DNA load in whole blood of 12000 copies/ml at day 5 after contact). She did not develop secondary complications of varicella and fully recovered. This patient showed a mitigated clinical course as compared to the IgG-negative immuno-compromised patient with a varicella infection who has not received varicella vaccination (viral loads up to 1000.000 c/mL can be seen). This mitigated course of the varicella is reflected by the relative low viral load and the lack of secondary complications.

**Determination of VZV-specific CD4\(^+\) T cells**

VZV-specific CD4\(^+\) T cells can be detected by intracellular cytokine staining, as we showed during memory responses of parents of children who experienced chickenpox as well as in some patients experiencing primary VZV infection (M.T.M. Vossen, The Journal of Infectious diseases, in press). Using this technique, we determined frequencies of VZV-specific CD4\(^+\) T cells in peripheral blood of the patients after varicella vaccination, by upregulation of CD69 and production of IFN-\(\gamma\) upon stimulation with VZV lysate (VZV ag). Virus-specific CD4\(^+\) T cells could not be detected in these children upon varicella vaccination, irrespective of seroconversion (Fig. 3A, n=8). Remarkably, these cells could not be detected upon vaccination-associated rash (Fig.3B; n=3), whereas they were clearly detectable upon development of varicella after encounter of subsequent wild-type VZV (Fig. 3C; n=1, VZV-specific CD4\(^+\) T cells 23 %).

**Virology**

In three of the eleven patients (pt. 1, 2, 11), VZV DNA loads were detectable upon vaccination in peripheral blood (n=3) (median of peak values 7500 copies/ml, range 40-10.000 copies/ml), within 6 weeks after vaccination (Table 4). No household exposures to the wild-type virus were documented during this period. These three patients all seroconverted in response to the vaccine. To determine whether the patients were contagious after varicella vaccination, a quantitative PCR was performed on throat swabs. Two patients had positive throat swabs (n=2) (Table 4). The nasopharyngeal cultures remained negative, indicating that the VZV DNA as detected in

<table>
<thead>
<tr>
<th>Table 4 Virology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
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</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

132
Figure 3 VZV-specific CD4+ T cell responses in relation to seroconversion.
VZV-specific CD4+ T cells (CD69"IFN-g") could not be detected in the follow-up of vaccinated patients who did generate VZV-specific IgG antibodies (n=8) (A). These cells could also not be detected in the follow-up of a vaccinated patient who developed a vaccine-related rash (n=3) (B), whereas they were clearly detectable within 14 days ("d.228") after appearance of the exanthem in a breakthrough case of natural chickenpox after exposure to wild-type VZV (n=1) (C). Where applicable, data shown are from one representative donor. Dot-plots are gated on CD4+ T cells. Numbers indicate percentages of CD69"IFN-g" cells (i.e. VZV-specific CD4+ T cells) within the CD4+ T cell gate. Control: in vitro stimulation with medium. VZV ag: in vitro stimulation with VZV lysate. d= days post varicella vaccination.
throat swabs of two of the patients did not result from infectious virus, but rather from VZV-infected lymphocytes residing in the throat. Most importantly, this demonstrated that the vaccinated patients did not shed any infectious virus and were therefore not contagious.

**Discussion**

This is the first cohort of pediatric oncology patients who receive the live attenuated varicella zoster vaccine in a relative early phase of the chemotherapy, without interrupting chemotherapy. The criteria that no steroids are given one week before and one week after vaccination, lymphocyte counts should be >700/mm³, and that the child has no bulky disease, were all met. Previous studies described the safety, immunogenicity and efficiency of the varicella vaccine in a large cohort of patients with leukemia in remission [14].

Remarkably, adverse effects of vaccination were limited in our cohort to a mild rash observed in three patients, accompanied by fever in two patients, mild gastro-intestinal problems in one patient, and mild respiratory complaints in one patient. The patients with prolonged rash were treated with aciclovir or famciclovir resulting in resolution of the rash. The occurrence of adverse effects in these patients did not correlate with the number of circulating lymphocytes at day 0 (data not shown). Seroconversion rates after one dose of vaccine was slightly lower in our cohort compared to the LaRussa cohort (73% vs. 82%). The induction of adaptive immunity may be hindered by chemotherapy. However, withdrawal of steroids during the period of vaccination most likely reduces this hindrance. More importantly, vaccination in such an early phase of treatment significantly increases protection from severe disease in this susceptible population. Seroconversion did not correlate with the age of the patients, the time between start of chemotherapy and vaccination, nor with the number of circulating lymphocytes.

Upon infection, varicella-zoster virus is believed to be contained by the concerted action of antibodies, CD4+ and CD8+ T cells and NK cells [3,7,16]. Although VZV-specific CD4+ T cells were undetectable by our assay upon vaccination, we believe that these cells were induced by vaccination since VZV-specific antibodies could be detected in the majority of these patients. VZV antigen is a lysate of VZV infected cells and contains a broad range of VZV-specific peptides. It is therefore unlikely that VZV-specific CD4+ T cells could not be detected in our assay due to absence of the immunodominant peptides.

Varicella vaccination protected one of the two patients with documented household exposure to wild-type VZV completely from development of varicella, and the other one from severe disease. Interestingly, the completely protected patient did not respond to varicella vaccination or to natural VZV infection, as classically defined by seroconversion. In our experience, as well as has been previously described in literature [6,22], unvaccinated cancer patients will develop severe courses of varicella upon household exposure. In the patient with household exposure who developed a mitigated course of varicella, virus-specific cells were clearly inducible within
14 days after appearance of the exanthem. These data suggest a suboptimal induction of adaptive immunity upon immunization, rather than primary vaccine failure. VZV-specific CD4+ T cells are probably induced upon vaccination, but their frequencies are too low to be detected in our assay. In this respect, it would be interesting to determine whether VZV-specific CD4+ T cells can be detected in healthy children upon vaccination, since it has been known that leukemic patients develop less pronounced VZV-specific T-cell responses upon VZV infection [16] and therefore, the expected frequencies of these cells would be higher in the healthy children than in our patients. VZV vaccination is not implemented in the Dutch vaccination program and it is therefore not possible for medical ethical reasons to perform these control experiments.

The incidence of herpes zoster still has to be determined in our cohort during follow-up. In immunized children with leukemia, a previous rash correlates with eventual development of zoster [13]. Special precautions were taken upon vaccination of these immunocompromised children to prevent infection of other children at risk. Although VZV DNA could be detected at borderline levels in only two sequential throat swabs of two patients in our total cohort, the cultures were negative. This indicates that these patients are not contagious upon vaccination, which is in accordance with the finding that v-Oka cannot be cultured from respiratory secretions [2]. However, the vaccinated patients that develop cutaneous lesions are infectious as proven by viral culture of vesicle fluids or scrapings. In this group of patients precautions for isolation need to be taken [2]. This study is promising in administering the varicella zoster vaccine during treatment without stopping chemotherapy. Administering the vaccine in an early phase of treatment will largely reduce the incidence of severe varicella during the complete course of chemotherapy. Larger cohorts of pediatric oncology patients will be required to determine the benefit of this strategy compared to the strategies studied so far.

Acknowledgements
The authors would like to thank Nelia Langeveld, Karla Peters, Annette Grummels and Jessica van der Sluijs for their excellent logistical and technical assistance.

Reference List


Appendix: Vasquez-score

Scale to Assess Severity of Illness

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash / Number of lesions</td>
<td></td>
</tr>
<tr>
<td>1-50</td>
<td>1</td>
</tr>
<tr>
<td>51-100</td>
<td>2</td>
</tr>
<tr>
<td>101-500</td>
<td>4</td>
</tr>
<tr>
<td>&gt;500</td>
<td>6</td>
</tr>
<tr>
<td>Character of lesions</td>
<td></td>
</tr>
<tr>
<td>Macular or papular</td>
<td>2</td>
</tr>
<tr>
<td>Mostly vesicular</td>
<td>4</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>4</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td>Temp 38.8-39.9°C</td>
<td>1</td>
</tr>
<tr>
<td>Temp &gt;40°C</td>
<td>3</td>
</tr>
<tr>
<td>Systemic signs</td>
<td></td>
</tr>
<tr>
<td>Pain in the back or abdomen</td>
<td>4</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>5</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>5</td>
</tr>
<tr>
<td>Subjective assessment (by nurse or physician)</td>
<td></td>
</tr>
<tr>
<td>Does not appear ill</td>
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</tr>
<tr>
<td>Appears moderately ill</td>
<td>2</td>
</tr>
<tr>
<td>Appears severely ill</td>
<td>5</td>
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</table>

Scale to Assess Severity of Illness

<table>
<thead>
<tr>
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<th>Number of Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity (total score in points)</td>
<td></td>
</tr>
<tr>
<td>Mild disease</td>
<td>&lt; 7</td>
</tr>
<tr>
<td>Moderately severe disease</td>
<td>8-15</td>
</tr>
<tr>
<td>Severe disease</td>
<td>&gt; 16</td>
</tr>
</tbody>
</table>

according to Marietta Vazquez et al, NEJM, 2001: 344: 956
Ungibonise yank' indlele
"you will show me the way"

Chapter

Discussion
The first part of this thesis discussed studies on predicting the course of infectious complications in the child with cancer, and the second part presented studies on preventing infection in which two systematic reviews were presented. Therefore the discussion will be divided in

a) Predictors of the clinical course of infection
b) Prevention of infection

At the end of the discussion an overview of the implications for clinical practice and proposals for future research will be given.

a) Predictors of the clinical course of infection

Chapters 3 and 4

With the increasing intensity of chemotherapy the cancer patient remains susceptible to infectious complications. In the introduction emphasis has been given to the various risks of the neutropenic patient for infection. Increased understanding of these various risks have led to risk assessment models which are now applied in adult patients\(^1/2\). These risk-assessment models have also been developed for children. Orudjev et al \(^3\) reported on all pediatric trials done on risk-assessment. Twenty-seven prospective trials were identified and five reviews. So far no internationally validated risk prediction rule is available for children. Deducted from the best available evidence one could define the low-risk prediction rule as follows: "The child with low-risk febrile neutropenia is clinically well and afebrile within 24-96 hours of therapy and has evidence of marrow recovery with a rising phagocyte count"\(^3\). Empirical therapy will be instituted in the hospital and decisions can be made to switch to oral antibiotic therapy after initial intravenous therapy. Prospective validation of the low prediction rule is necessary prior to implementing this rule in pediatric oncology practice. Next to clinical parameters biochemical parameters can be used as diagnostic tools to predict the course of the severity of an infection\(^4\). In this thesis we concentrated on predicting the course of one severe complication in oncology patients, i.e. neutropenic enterocolitis.

In Chapter 4 we presented a prospective single center study gaining insight in the incidence and pathogenesis of neutropenic enterocolitis in pediatric oncology patients. In this study twenty-five patients were included (mean age 7.1 years) with suspected neutropenic enterocolitis. Eight patients (32%) needed intensive care treatment, 3 (12%) patients died. Predictors of a severe clinical course of the enterocolitis were an increased serum IL-8 (>1000 pg/mL) and an increased serum CRP (>150 mg/L). Relative risks for admission to ICU was 11.3 (95% CI 1.6 to 77.9) for elevated IL-8. Therefore IL-8 on the first day of suspected neutropenic enterocolitis can be used as a predictive marker.

In future studies it is important to try and prevent this severe complication.

If you can prevent severe mucositis, this will most likely lead to less gastrointestinal damage and therefore a decrease in the incidence of neutropenic enterocolitis.
A phase III cross over designed study has recently started in 2 pediatric oncology centers (Sophia Children’s hospital, Rotterdam and Emma Children’s Hospital, Amsterdam) administering Transforming Growth factor β (TGF-β) as a mouthwash and added to the food as an oral solution to pediatric oncology patients who are expected to develop a severe mucositis on the basis of the chemotherapy given (Principal investigator Prof. R. Pieters: Sophia Children’s Hospital, Rotterdam). TGF-β is a growth factor present in many mammalian tissues. In vivo studies have shown that the mucous membranes stabilize after adding TGF-β, because TGF-β can inhibit the proliferation of epithelial cells. Chemotherapeutic drugs target rapidly proliferating cells. The capacity of TGF-β to down regulate the proliferation of the oral and gastro-intestinal epithelial cells makes this growth factor of interest in possibly decreasing the chance for severe mucositis in oncology patients. This can consequently lead to decreasing the incidence of neutropenic enterocolitis.

Another way to improve the human intestinal epithelial barrier function might be the introduction of probiotics in an early stage of the treatment. Probiotics have been defined as living organisms in food and dietary supplements which upon ingestion improve the health of the host beyond the inherent basic nutrition. In neonatal necrotizing enterocolitis interest has been shown in the administration of Bifidobacterium infantis and lactobacillus acidophilus. Lactobacillus appears to have protective immunomodulating properties inducing a Th2 response. And Lactobacillus have the ability to inhibit the adhesion of pathogenic bacteria to the intestinal wall. The pathogenesis however in cancer patients is different, the interaction of chemotherapy, antibiotics and damage to the gastro-intestinal barrier may be more difficult to influence with probiotics, this makes the application of probiotics uncertain.

It will not always be possible to prevent neutropenic enterocolitis. Therefore attention needs to be given to the patients who are predicted to develop a severe neutropenic enterocolitis using biochemical markers like IL-8 and CRP. Patients have a higher chance to recover if granulocytes are present. A future intervention study could focus on administering granulocyte-transfusions. For many years this has not been advocated because of the limitations in collecting adequate doses of leukocytes from healthy donors by steroid mobilization. The development and use of granulocyte colony stimulating factors to stimulate normal donors has generated renewed interest in granulocyte transfusions. The yield of leukocytes collected from normal donors is high, this could improve outcomes in patients with severe infections and severe neutropenia. A Cochrane systematic review has recently been published on the use of granulocyte transfusions in neonatal sepsis. Four RCT’s were identified. From these trials there is inconclusive evidence that the use of granulocyte transfusions leads to a reduction in morbidity and mortality. A well designed randomised clinical trial is necessary to establish granulocyte transfusions as a viable therapeutic modality in the treatment of severe bacterial and fungal infections in patients who are deeply neutropenic.
MBL and prediction of the course of infections

If biochemical markers are going to be used in risk assessment models it will become very important to understand the mechanisms of the innate immune system. This immune system plays a critical role in the first few days of infection. Why some individuals always develop infections and others don’t might be linked to genetic differences. For instance MBL deficiency has been linked to longer febrile neutropenic episodes, and in other studies to more severe infections.

In Chapter 3 a pilot study is presented evaluating the role of mannann-binding lectin deficiency (MBL) in pediatric oncology patients with neutropenic fever. Twenty four patients were prospectively followed during an episode of febrile neutropenia. The incidence of MBL exon-1 gene mutations and MBL-deficiency (<800 µg/L) was 32.5%. However, no correlation was found with either the severity or frequency of the infections, although a trend was found towards a longer duration of neutropenia in the MBL-deficient group of patients. If we used the cut-off value of 1000 µg/L (calculated with a ROC curve) there was a trend towards more bacteremia’s 37.5% in the MBL insufficient group compared to 13.3% in the MBL sufficient group. We evaluated a small cohort of patients, and results might change if the cohort is extended. Kilpatrick et al. found no relation with severity or frequency of infections in a large group of adult oncology neutropenic patients. These patients might be too neutropenic, therefore the effect of MBL deficiency could be overshadowed. This might also be the case in our studied cohort. Therefore it is important to extend the cohort to ultimately define a group who will benefit most from substituting MBL, possibly the patients who receive their induction-period of chemotherapy.

The possibility is already present to substitute MBL. In 1995 the Statens Serum Institut (SSI, Copenhagen, Denmark) began developing MBL concentrated plasma product, from pooled human plasma for the treatment of patients with frequent infections associated with MBL-deficiency. A phase I trial has been completed investigating the safety and pharmacokinetics of MBL in 20 adult MBL deficient volunteers (see product information SSI, Denmark, Kopenhagen). This was completed without side-effects being reported and good tolerability. Now a small phase II trial has been designed and approved by the ethical committee to find evidence in 12 MBL-deficient pediatric oncology patients for the correct prediction of plasma levels of MBL, to confirm the dosage regimen needed to reach the required MBL plasma level, and reconfirm the safety and lack of side-effects (Principal Investigator: Prof. T. Kuipers. CLB Amsterdam). Together with the data from the extended observational cohort study, this will gather the data needed to decide on a prospective randomised placebo-controlled phase III efficacy study in pediatric oncology patients with MBL replacement therapy.
b) Prevention of infection

Chapters 5, 6 and 7

Long-term tunnelled central venous catheters (TCVC) are increasingly used in oncology patients. Infections are a frequent complication of TCVC (Groeger, 1993, 206; Press, 1984, 189). These infections are mostly caused by Gram-positive bacteria (Mermel, 2000, 391). The aim of the systematic review as described in Chapter 5 was to evaluate the efficacy of antibiotics in the prevention of early Gram-positive TCVC infections. MEDLINE, EMBASE, and the Cochrane Controlled Trials Register were searched up to July 2003. All randomized controlled trials (RCT) evaluating prophylactic antibiotics prior to insertion of the TCVC, and RCT's evaluating the combination of an antibiotic and heparin to flush the TCVC were included. Both pediatric and adult oncology patient trials were selected. A total of 9 trials with 529 patients were included. Four reported on vancomycin/teicoplanin prior to insertion of the TCVC compared to no antibiotics, and 5 on flushing of the TCVC with a vancomycin/heparin solution compared to heparin flushing only. Both antibiotics prior to insertion of the catheter compared to no antibiotics and flushing the TCVC with antibiotics and heparin showed a significant reduction in the number of Gram-positive TCVC infections. The respective Odds ratio's were [OR]=0.46: 95% confidence interval 0.24-0.91 and [OR]=0.43, 95% CI 0.21-0.87). In oncology patients who need a TCVC and are at high risk for Gram-positive infections it is justified to use the above interventions. This should be implemented in clinical practice, especially in patients where the expected baseline infection-rate exceeds 10%, such as hematological patients during induction-phase of therapy and autologous and allogenic transplant patients.

Other preventative strategies to reduce catheter-related infections have so far been restricted to short-term non-tunnelled catheters. Studies involving antimicrobial/antiseptic impregnated catheters have all been performed in adult patients using non-cuffed catheters. Two meta-analyses have been performed on the use of chlorhexidine/silver sulfadiazine on the external luminal surface of the catheter 16,17. The studies analyzed showed a reduction in catheter-related bloodstream infections with a relative risk of 0.4. The benefit was realized in the first 14 days of placement of the catheter. Resistance to the chlorhexidine-silver sulfadiazine catheter has not been demonstrated in clinical studies. Newer generations of these catheters now coat the external surface with three times the amount of chlorhexidine and silversulfadiazine and the internal surface is only coated with chlorhexidine. Results have shown that prolonged anti-infective activity provides improved efficacy in preventing infections 18. These catheters may be recommended in patient populations in which the infection-rate exceeds 3.3 per 1000 catheter days 19. A more recent alternative is to impregnate catheters with minocycline /rifampin. This has been shown to be 12 x more effective than the first generation chlorhexidine/sulfadiazine impregnated catheters 20, however no trials have been done comparing this catheter to the second generation chlorhexidine /silversulfadiazine ones. This catheter does however seem
promising in patients with an expected duration of the catheter of 3 weeks. So far these strategies can not be used in long-term tunneled catheters.

Other ways to prevent infection in cuffed catheters is the use of ionic silver in subcutaneous collagen cuffs attached to the central venous catheter\(^2^1\). The ionic silver provides antimicrobial activity and the cuff is a mechanical barrier to the migration of micro-organisms along the external surface of the catheter. However, this model is not very effective in reducing catheter related bloodstream infections in catheters that need to be in place for at least 3 weeks\(^2^2\).

Segura et al\(^2^3\) introduced a new hub model to decrease endoluminal catheter contamination and catheter-related sepsis. This hub consists of a closed chamber containing 0.2 ml 3% iodinated alcohol (6 mg iodine). In this RCT the incidence of catheter related sepsis decreased with the use of this system in short-term catheters. No trials have so far been performed with this new hub in long-term tunnelled central venous catheters. Features of concern for long-term tunneled central venous catheters are the iodine stability and the long-term hub concealment.

An alternative method of treatment of central venous catheter related infections is the use of antibiotic-lock therapy, first reported by Messing et al\(^2^4\), this is the introduction of a concentrated antibiotic solution into the catheter to "dwell" for an extended time. In this study 90% of the infections were treated successfully. So far only open trials have been done in tunnelled catheter-related bacteremia, with or without additional parenteral antibiotic therapy. With antibiotic-lock more catheters were salvaged. For instance vancomycin has been used in a dosage of 1-5 mg/mL mixed with 50-100U of heparin and 2-5 mls are instilled into the catheter to "dwell" for 12 hours\(^2^4,2^5\). The duration of treatment has varied, but it is most often 2 weeks\(^2^4,2^6\). Up to date no RCT's have been performed in patients with cancer. Therefore future trials are needed and antibiotic lock therapy should not be common practice as yet.

A further aspect in preventing infections in oncology patients was reviewed in Chapter 6. In this chapter a systematic review is presented to assess the evidence for the effectiveness of selective gut decontamination (SDD) to decrease bacteremia and infection-related mortality during neutropenic episodes in oncology patients. Medline, Embase and the Cochrane Library issue 2, 2002 were searched. The main outcome was the number of patients with documented bacteremia's (Gram-negative or Gram-positive bacteremia) and infection related mortality. A total of 21 studies met the inclusion criteria. The incidence of Gram-negative bacteremia's significantly decreased and showed an OR of 0.39 (95% CI 0.24-0.63). Infection related mortality due to bacterial causes decreased with the use of SDD, an OR of 0.49 was seen (95% CI 0.27-0.88). In conclusion this systematic review has shown that TMP/SMZ or quinolone based SDD regimen started before the onset of neutropenia reduces Gram-negative bacteremia and infection related mortality in neutropenic oncology patients. From our results we highly recommend the use of TMP/SMZ or quinolone prophylaxis to cancer patients with a high baseline risk for infections, such as patients with hematological malignancies, autologous and allogenic bone-
marrow transplant patients and solid tumour patients who have an expected neutropenia of at least 7 days.

A similar debate has been held in the ICU as to whether or not antibiotic prophylaxis should be used to prevent or decrease respiratory tract infections. Although the endpoints in ICU patients are different, namely respiratory tract infections and the method of SDD in these patients differs from oncology patients, also in this ICU group the lack of a standard protocol and insufficient numbers have made it difficult to derive meaningful conclusions from individual clinical trials. Recently a well designed randomised controlled trial was performed in an ICU setting with the inclusion of 934 patients, assessing the effect on ICU mortality, hospital mortality and the acquisition of resistant bacteria. The SDD given was polymyxin E, tobramycin, and amphotericin B combined with a 4 day course of intravenous cefotaxime. In the SDD group 15% of patients died in ICU versus 23% in the control group. Resistant Gram-negative or Gram-positive organisms were found in 16% of the SDD patients and in 26% of the control patients. Their conclusion is, SDD can decrease ICU and hospital mortality and colonization with resistant Gram-negative aerobic bacteria in a setting with low prevalence of vancomycin-resistant enterococcus and methicillin-resistant Staph. aureus. At the same time a Cochrane review was published addressing the same question. In the 16 included trials that tested a combination of topical and systemic antibiotics, there was a significant reduction of both respiratory tract infections (OR 0.35 CI 0.29-0.41) and total mortality (OR 0.80 CI 0.69-0.93). The design of the review did not allow conclusions to be drawn on resistance data. However, the data of this systematic review and the large RCT provide evidence that this strategy should be implemented in clinical ICU practice.

To improve our understanding of other supportive care issues systematic reviews on the research performed are useful. A problematic aspect in pediatric oncology even in multi-centre trials is the number of patients involved in the trial. Because the size of the group is small it is often difficult to detect a significant difference in the effects of two therapies given. Therefore systematic reviews should be considered essential tools for researchers and health-care workers. Systematic reviews allow a more objective appraisal of the available evidence and contribute to resolve uncertainty when original research, reviews and editorials disagree. Meta-analysis, if possible, reflects a weighted average of the results in which larger trials have more effect than smaller trials. If there is no heterogeneity between the included studies an overall effect can be presented with a confidence interval. Systematic reviews can then contribute to considerations regarding the applicability of the study results. Systematic reviews are also important to define areas in which further trials are warranted. The quality of these reviews will only improve if groups collaborate as is advocated in the Cochrane Collaboration Method group. Until now pediatric oncology was reviewed by the Cochrane Orphan group, but fortunately as from 2004 pediatric oncology will have its own Cochrane Childhood Cancer Review group (Dr. L.C.M. Kremer, EKZ/AMC Amsterdam, in collaboration with the Dutch Cochrane Center).
In Chapter 7 we presented a prospective study on vaccinating IgG-negative children with cancer in an early stage of their disease with the live-attenuated VZV vaccine. We achieved 72.7% seroconversion after the first dose. This is the first cohort of pediatric patients with cancer who received the VZV vaccine without interrupting the chemotherapy and the first cohort where the vaccine is introduced in a relative early phase of the chemotherapy. This study demonstrates that it is possible to administer this live attenuated vaccine during chemotherapy. Although the seroconversion is somewhat lower than in the study of LaRussa et al 30 who vaccinated 509 leukemic children in the maintenance phase, with suspension of the chemotherapy, our study is promising in decreasing the incidence of varicella zoster infection during chemotherapy treatment and most important decreasing complications due to varicella zoster infection.

One option to decrease varicella infection is to immunize all children, as part of a routine vaccination programme. This is now done in the USA.

In the USA varicella vaccine has been licensed since 1995. Vaccine coverage assessed by the 2000 National Immunization survey in the USA was 68%. In order to achieve disease control vaccination coverage of over 90% is needed 31. It has been proven that the incidence of admissions for varicella and complications thereof have decreased significantly since the introduction of the routine varicella immunization programme. The future lies in total prevention of varicella by achieving an adequate vaccination coverage. Before this coverage has been achieved it is extremely important to recognize the “at high risk” patient (immuno compromised patients and pregnant women) who are at even higher risk of complications because of the relative low herd immunity 32. Furthermore other countries do not find vaccinating all children to be cost-effective. The risk of complications in “normal” children is so low, that this does not warrant routine immunization. Thus in both these groups it will be of extreme importance to offer seronegative immunocompromised patients varicella protection as soon as possible after diagnosis.

Our cohort of patients will be extended obtaining more data on time to seroconversion, adverse effects and seroconversion after a second dose of vaccine.

In patients acquiring varicella infection or herpes zoster infection oral treatment has improved. It is known that aciclovir has poor oral bioavailability and therefore should be dosed at least 5x per day. Newer drugs are available, valaciclovir and famciclovir. These drugs are rapidly converted to aciclovir and the bioavailability is 3-5x higher than that of oral aciclovir in humans 33. This results in improved benefit, and most likely improved compliance. These drugs are not registered for pediatric use yet. Future trials should focus on the efficacy of these newer drugs in immunocompromised children.

**Overall conclusion**

Many aspects of preventing and predicting infections in pediatric oncology patients have been addressed in this thesis. Proposals for future trials have been presented. The results of future
prospective trials and the evidence acquired from systematic reviews, will allow the best available treatment of infectious complications to be applied to our pediatric oncology patients.

Reference List


Implications for clinical practice
and future trials
Implications for clinical practice

IL-8 and CRP should be measured on day 1 of clinical suspect neutropenic enterocolitis to stratify patients who have a high chance of being admitted to ICU and those at low risk of a severe course of neutropenic enterocolitis. In the high risk group inotropic support should be given in an early stage.

In patients who need a tunnelled central venous catheter, and who are at high risk of infections it should be implemented to give Gram-positive antibiotic cover prior to insertion of the catheter, or to flush the catheter with the combination of an antibiotic and heparin.

In patients at high risk of infections during neutropenic episodes SDD (TMP/SMZ or quinolones) should be used started prior to the onset of neutropenia.

Routine vaccination of VZV IgG-negative pediatric oncology patients is not yet warranted.

Implications for future research

The prevention of mucositis will ultimately lead to the prevention of neutropenic enterocolitis. Ongoing phase III cross over designed trial administering TGF-β (Transforming growth factor) as a mouth wash and added to the feeds as oral solution, to pediatric patients who are expected to develop a severe mucositis on the basis of the chemotherapy given.

Intervention trial in the predicted high-risk group of patients for neutropenic enterocolitis. If the high-risk group of patients is identified in an early stage of the disease, a RCT needs to be designed to administer granulocyte-transfusions to the high-risk group of patients, and to evaluate the severity and outcome of the neutropenic enterocolitis.

To extend the cohort of patients in which genotyping of MBL and serum levels of MBL are followed during febrile neutropenic episodes, gaining insight in the relation of MBL-deficiency and severity of infections. This allows us to identify the group of patients who might benefit most from MBL-substitution.

In vivo studies on tunnelled catheters to develop a coating system that will lead to reduction of catheter related infections for the entire duration of the catheter, without developing resistant organisms.
With our systematic review it was shown that Gram-positive catheter related infections can be decreased by using antibiotics prior to insertion of the catheter or flushing the catheter with an antibiotic and heparin. In the treatment of Gram-positive catheter related sepsis antibiotic lock therapy has proven to be of benefit in immuno-competent patients. No trials so far have been performed in the immuno-compromised pediatric patient. Therefore a randomised controlled trial needs to be performed, to apply antibiotic-lock therapy with or without systemic therapy to treat the Gram-positive catheter related infection.

Our preliminary study on vaccinating IgG-negative pediatric oncology patients in an early stage of their disease showed a high seroconversion-rate and is therefore promising. Before implementing this vaccination routinely a large trial on vaccinating IgG-negative VZV pediatric oncology patients early during treatment is necessary to gain further insight in immunogeneity, efficacy and adverse effects.
the child is my best friend.
General overview

Infections in patients with cancer remain an important problem. Therefore this thesis focused on prediction and prevention of infectious complications in patients with cancer.

The first part of this thesis focuses on immunological parameters as possible predictors of severity of infection, such as the significance of mannose-binding lectin in infectious complications, and the role of cytokines in predicting the severity of the clinical course of neutropenic enterocolitis.

The second part of the thesis focuses on prevention of infections. Three aspects will be discussed: 1) prevention of Gram-positive catheter-related infections; 2) prevention of bacteremia during episodes of neutropenia using selective decontamination of the digestive tract (SDD) 3) prevention of varicella in varicella-zoster virus (VZV) IgG-negative children with cancer.

By gaining more insight in predicting and preventing infectious complications during the treatment of cancer patients, care and management of infections in this group of patients will improve.

In Chapter 1 a general overview is presented on epidemiological aspects, causes and complications of infections in oncology patients. Two infections which can cause serious complications are discussed in more detail. These are neutropenic enterocolitis, bowel wall inflammation during deep neutropenia, and varicella zoster (VZV) infection which can lead to serious complications in patients who are neutropenic and not protected against VZV.

In Chapter 2 a retrospective study is presented on risk-factors for infection in a single oncology unit in South-Africa. Limited data are available on infectious complications in pediatric oncology patients in countries, where most patients come from rural area’s. Most children are admitted in an advanced stage of their disease. The poor housing circumstances, the long travel distances and therefore the long duration of hospitalization might influence the risk for infection. Between 1991 and 1995 all data on positive blood-cultures in pediatric oncology patients were collected, after which the medical records were studied. 200 separate episodes of bacteremia were recorded in 83 patients. Of these 200 episodes 83 were first bacteremic episodes. Of the 83 first bacteremic episodes 8 ended in death of the patient. Mainly Gram-negative organisms were seen, resistant Acinetobacter Baumanii played a major role. In the following bacteremic episodes fungal organisms were more important, mainly Candida parapsilosis. A risk-factor for infection was the presence of a tunneled central venous catheter (CVC). The mean incidence of catheter related infections was 3.3 episodes per 1000 catheter days. Of all fungal infections 64% were reported in children with a CVC. Striking finding was the high incidence of Gram-negative organisms and a relative low incidence of Gram-positive organisms. Cause of the increased incidence of resistant Acinetobacter Baumanii and fungal organisms is most likely the long hospital-stay of these patients (mean 83 days) which could lead to higher rates of
Chapte rr  10

colonization and indirect transmission between patients. Gaining insight in infectious complications in this group of patients allows us to consider intervention trials to reduce Gram-negative and fungal infections.

Chapter 3 and 4 focus on prediction of the severity of the course of the infection

In Chapter 3 a pilot study is presented evaluating the role of mannan-binding lectin deficiency (MBL) in pediatric oncology patients with neutropenic fever. MBL is a serum protein produced in the liver that plays a critical role in the innate immune response. 25% of the normal population is MBL deficient. This deficiency is recognized clinically in patients with co-existing immunodefects. Possibilities are now available to start replacement therapy with MBL. A prospective study needs to gain insight in the patients who will benefit most from replacement therapy with MBL. Over a period of 8 months all pediatric oncology patients who were expected to become neutropenic were considered for inclusion. From these patients MBL genotyping and MBL serum levels were performed. The clinical outcome parameters looked at were duration of fever, duration of neutropenia, signs of septicemia, intensive care admission and mortality due to infection. Forty patients were genotyped of which 24 (60%) had a febrile neutropenic episode. The incidence of MBL exon-1 gene mutations was found to be 32.5% and the incidence of MBL-deficiency (<800 ßig/L) was 32.5%. No correlation was found with either the severity or frequency of the infections, although a trend was found towards a longer duration of neutropenia in the MBL-deficient group of patients. Most of the included patients were not "newly diagnosed" patients but had been on chemotherapy at least several months (median 8 months, range 0-59 months). 70% of all patients presented with a severe neutropenia (<100x10^9/L). Because of the severe neutropenia the effect of MBL deficiency might be completely overshadowed. We will extend this cohort with "newly diagnosed" patients to identify the subset of patients who might benefit from mannan-binding lectin substitution.

In Chapter 4a a case-report is presented describing 2 patients with an enterocolitis. In the first patient this was caused by Clostridium difficile and in the second patient no organism was found, but the pathological severity resembled a typhilitis-like picture. In Chapter 4b a prospective study is presented of 25 pediatric oncology patients admitted between 1998 and 2002 with the clinical suspicion of neutropenic enterocolitis. Inclusion criteria were fever, neutropenia, abdominal pain and diarrhea. 8 of these children were admitted to ICU. On day 1,3 and 7 clinical parameters and laboratory parameters were done, this also included a rectoscopy on day 1 and immunological parameters (CRP, IL-8, IL-6 and IL-10) on day 1,3 and 7. The main findings of the study showed that clinical parameters, rectoscopy, and blood or stool-cultures did not predict the severity of the course of neutropenic enterocolitis. Immunological parameters were better predictors. If CRP on day 1 >150 mg/L then the chance of admittance to ICU was 6.4x higher than in children with the same complaints and a lower CRP. For IL-8 this difference
was even more significant. The chance of ICU admission was 11.3 x higher if IL-8>1000 pg/mL, 6 out of 7 ICU admissions had a level>1000 pg/mL, compared to 2 out of 16 non-ICU patients. In this cohort of patients IL-8 was considered the best predictor of severity of the clinical course of neutropenic enterocolitis. Future trials will be directed at intervention of the high risk patients, possibly by starting inotropics early, or giving granulocyte-transfusions.

**Chapter 5, 6 and 7 focus on prevention of infection**

In **Chapter 5** a systematic review is presented on the use of prophylactic antibiotics to prevent Gram-positive catheter-related infections. One of the main complications of a tunnelled central venous catheter is the risk for infection. Most catheter-related infections are caused by Gram-positive organisms. There is no consensus on the use of prophylactic antibiotics to prevent Gram-positive catheter-related infections. Therefore this review was done. All randomized trials on both adult and pediatric oncology patients between 1966 and 2003 were searched. 33 trials were identified and 9 could be included. 4 trials reported on vancomycin or teicoplanin before insertion of the catheter, and 5 trials reported on flushing the catheter with a combination of vancomycin and heparin. In the 4 trials reporting on antibiotics before insertion of the catheter, there were 17 patients with a Gram-positive catheter related sepsis in the group receiving antibiotics (n=95) and 30 patients in the control group (n=92). The Odds ratio found was 0.46 (95 % CI 0.24-0.91). In the 5 trials reporting on flushing the catheter with vancomycin and heparin, there were 13 patients with a Gram-positive catheter related sepsis in the group receiving the flushing method of vancomycin and heparin (n=153) and 31 patients in the control group (n=189). The Odds ratio found was 0.43 (95% CI 0.21-0.87). The conclusion is that both interventions in a high-risk patient are beneficial. To apply the above strategies will depend on the base-line infection risk of the patient. Patients with hematological malignancies and bone-marrow transplant patients will benefit from prophylactic antibiotics prior to insertion of the catheter or flushing the catheter with the combination of vancomycin and heparin to prevent Gram-positive catheter-related infections.

In **Chapter 6** a systematic review is presented on the efficacy of the use of selective gut decontamination in oncology patients. This strategy has been known since the early 70's. Gram-negative bacteremia's are decreased with effective use of the SDD.

No consensus on its use has been achieved, mainly because the effect on decreasing infection-related mortality was not known, and the fear for creating resistant strains. A systematic review was performed including all randomized trials between 1966 and 2002. A total of 21 trials met the inclusion criteria. Seventeen trials compared SDD (quinolones or Trimethoprim/sulfamethoxazole (TMP/SMZ)) to no SDD, and 4 trials compared quinolones to TMP/SMZ. The incidence of Gram-negative bacteremia's significantly decreased. There were 59 patients in the control group with a Gram-negative bacteremia (n=517) and in the SDD group
25 patients (n=530). The OR was 0.39 (95% CI 0.24-0.63). Quinolone-based regimens showed a stronger reduction in Gram-negative bacteremia's, and TMP/SMZ based regimens showed a stronger reduction in Gram-positive bacteremia's. Infection related mortality due to bacterial causes decreased with the use of SDD. There were 23 patients who died of an infectious cause in the SDD group (n=564) and 39 patients in the control group (n=551). An OR of 0.49 was found (95% CI 0.27-0.88).

From our results we consider that TMP/SMZ or quinolone prophylaxis should be administered to cancer patients with a high baseline risk for infections, such as patients with hematological malignancies, autologous and allogenic bone-marrow transplant patients and solid tumor patients who have an expected neutropenia of at least 7 days.

In Chapter 7 the focus is on prevention of varicella zoster infection in pediatric oncology patients. One patient is presented in Chapter 7a. Until now many trials have been performed on administering live attenuated varicella vaccine to this group of patients. In the trials performed chemotherapy was stopped one week before and two weeks after the vaccination. Seroconversion occurred in 95% of vaccinees after 2 doses of vaccination. In Chapter 7b a prospective pilot-study is presented. This is the first cohort of pediatric oncology patients who received the attenuated live varicella zoster vaccine in a relative early phase of the chemotherapy without interrupting the chemotherapy. Eleven patients with either a hematological malignancy (n=8) or a solid tumor (n=3) were vaccinated with VZV vaccine during chemotherapy. Seroconversion occurred in 8 of the 11 patients (72.7%). The only adverse effects consisted of a mild rash (50-200 lesions). In none of the patients chemotherapy needed interruption. This study demonstrates that it is feasible to administer VZV-vaccine in an early stage of chemotherapy without interruption of the chemotherapy. This will largely reduce the incidence of severe varicella during the complete course of chemotherapy. Larger cohorts of pediatric oncology patients will be required to determine the benefit of this strategy compared to the strategies studied so far.

In the closing Chapter 8 all the results found on predicting the course of severity of infection and preventing infections are further discussed and proposals for future trials are given; mannan-binding lectin substitution in MBL-deficient oncology patients, intervention trials on high risk neutropenic enterocolitis patients such as the administration of granulocyte transfusions or substitution of endothelial growth factor. Trials on VZV vaccine achieving a higher conversion-rate in oncology patients without stopping chemotherapy, determining the time to boost these patients, and gaining more insight in response to household contacts after vaccination. Implementation of systematic reviews in clinical practice
Thanda ntwana kube kube nguna phakade
"the love of the child may it endure unto eternity"

Chapter

Samenvatting
Het overlevingspercentage van kinderen met kanker ligt rond de 70%. Tijdens de behandeling vormen infecties nog steeds een groot probleem. Het eerste deel van het proefschrift beschrijft onderzoek om de ernst van een infectie te voorspellen. In het tweede deel van het proefschrift wordt aandacht besteed aan methoden om infecties te voorkomen.

In Hoofdstuk 1 wordt een algemeen overzicht gegeven over infecties en de complicaties hiervan in de oncologie. Bij oncologische patiënten verdacht van een infectie wordt er bij 12-17 % daadwerkelijk een bacterie of een schimmel gevonden worden. Bij de volwassenen sterft 4-6% van de patiënten ten gevolge van een infectie, bij kinderen is dit 0-6-1%. De organismen, welke het meeste voorkomen zijn Gram-positieve (bv coagulase negatieve staphylococcen), maar de meest ernstige infecties die voor kunnen komen zijn de Gram-negatieve organismen en schimmel infecties. Het is van groot belang om bij kinderen met koorts in aplasie niet te wachten met de behandeling voor infectie, maar hier zo spoedig mogelijk aan te beginnen. Het is bekend dat er verschillende risicofactoren zijn welke leiden tot het ernstig of minder ernstig verlopen van deze koorts in aplasie episode. Zowel klinische tekenen als bepaalde laboratorium waarden kunnen het verloop en de ernst van deze infecties voorspellen. Hierop is het eerste deel van het proefschrift gericht.

In Hoofdstuk 2 wordt een onderzoek beschreven naar risico-factoren voor infecties in kinderen behandeld voor kanker in Zuid-Afrika. Dit zijn kinderen die vaak in een laat stadium van hun ziekte worden opgenomen en waarbij de opname duur veel langer is dan in westere landen vanwege de lange reisafstanden naar hun huis. Tussen 1991 en 1995 werden alle positieve bloedkweken (dwz als er een bacterie of een schimmel in het bloed werd aangetoond) bij 83 kinderen met kanker nagekeken. Er werden 200 positieve bloedkweken vastgesteld, waarvan 83 positieve bloedkweken als eerste episode werden geregistreerd. Het betrof 70% Gram-positieve organismen, 20% Gram-negatieve organismen en 10% schimmel-infecties. Van de 83 eerste bacteriële episoden eindigden 8 in de dood van het kind. Hier speelden Gram-negatieve infecties een belangrijke rol. In de volgende episoden van positieve bloedkweken speelden schimmel-infecties een voorname rol. Een duidelijk risicofactor in deze groep patiënten was het aanwezig zijn van een centraal veneus getunnelde catheter. Alle kinderen hadden een Broviac of Hickman catheter (een chirurgisch ingebrachte lijn die gebruikt wordt voor het toedienen van de chemotherapie, vocht en bloedproducten). Van alle schimmel-infecties kwamen 64% voor in kinderen met een Broviac/ Hickman catheter. Verder was opvallend dat er veel Gram-negatieve infecties voorkwamen in deze kinderen met getunnelde lijnen, vooral infecties die resistent waren tegen de meeste antibiotica. Oorzaak van zowel de schimmel-infecties als de Gram-negatieve infecties was waarschijnlijk de langdurige hospitalisatie van deze kinderen. Inzicht in risico-factoren, welke organismen een rol spelen en welke organismen tot de dood van de patient leiden maakt een betere begeleiding van de oncologische patient tijdens een infectie episode mogelijk.
In **Hoofdstuk 3** wordt er onderzoek gedaan naar de rol van het afweer systeem voor het ontwikkelen van een ernstige infectie. De afweer bestaat uit een vroege afweer die werkt in de eerste dagen van een infectie en een afweer die daarna begint, waar al een soort geheugen is opgebouwd voor het vechten tegen de infectie. Mannose bindend lectine (MBL) is onderdeel van dat deel van het immuun-systeem dat een rol speelt bij de vroege opvang van een infectie. Het is een eiwit dat in de lever wordt gemaakt en dat bij 25% van de bevolking verminderd aanwezig is. Als deze mensen een infectie krijgen dan kunnen zij hier toch goed tegen vechten omdat de rest van het afweersysteem normaal functioneert. Maar als er nog een andere afweerstoornis bij komt, zoals in patiënten met kanker, dan is het mogelijk dat in mensen met een tekort aan MBL er meer ernstige infecties kunnen ontstaan. Er zijn 6 studies verricht zowel bij volwassenen als bij kinderen waarin gekeken wordt naar de relatie tussen het verminderd aanwezig zijn van het MBL en de ernst van de infecties. Tot nu toe was er nog maar één studie gedaan in kinderen en die studie liet zien dat in die kinderen een langere duur van de neutropenie werd aangetoond. Om meer gegevens over kinderen te verkrijgen is er door ons in het AMC een studie verricht over een periode van 8 maanden (februari 2003 – oktober 2003). Hierbij werd van alle kinderen die behandeld werden voor kanker het MBL gen en de MBL waarde in het bloed bepaald. Er werd gekeken naar de ernst van de infectie en de duur van de neutropenie. Er werd bij 40 kinderen een MBL genotype bepaald. Van deze kinderen had 32.5% een afwijkend genotype. Dit komt overeen met wat in de literatuur gevonden wordt. Van deze 40 kinderen ontwikkelden 24 koorts terwijl de afweercellen laag waren. Er kon geen verschil worden aangetoond in ernst van de infecties bij de groep die een tekort aan het eiwit had vergeleken met de groep die dit tekort niet had. Het leek alsof de groep met een MBL tekort een langere duur van de neutropenie ondervond, net zoals in de eerdere studie. Het aantal patiënten zal uitgebreid worden om meer te zeggen over de voorspellende waarde van een MBL tekort bij kinderen met kanker.

**Hoofdstuk 4** besteedt aandacht aan een ernstige infectieuze complicatie in patiënten met kanker, neutropene enterocolitis, (een ernstige ontsteking van de darm). In **Hoofdstuk 4a** wordt het ziektebeloop van 2 patiënten beschreven. Door tijdige herkenning van dit ziektebeeld en het bieden van intensieve zorg konden beide patiënten goed behandeld worden en hadden beiden daarna geen klachten meer. In **Hoofdstuk 4b** wordt een studie beschreven waarbij tussen 1998 en 2002 alle kinder-oncologische patiënten werden ingesloten met koorts, lage afweer cellen, buikpijn en diarrhee klachten. Dit waren 25 kinderen, waarvan 8 kinderen op de intensive care (IC) opgenomen moesten worden. Op dag 1, 3 en 7 van de klachten werden zowel klinische als laboratorium gegevens verzameld. Alle kinderen ondergingen een rectoscopie op de eerste dag van de klachten (waarbij er met een lampje in het laatste deel van de darm wordt gekeken en er tegelijkertijd stukjes darmweefsel kan worden verwijderd om onder de microscoop te bekijken), en bij alle kinderen werd bloed-onderzoek verricht. De belangrijkste
resultaten van het onderzoek toonden dat de rectoscopie geen voorspellende waarde had voor de ernst van het verloop van de neutropene enterocolitis, ook bloed en onlasting kweken konden niet voorspellen welke kinderen een ernstiger klinisch beloop zouden tonen, maar de immunologische parameters wel (bepaalde waarden van het afweersysteem). De belangrijkste waarde was het interleukine 8 (IL-8). De kans op een ernstig verloop van een neutropene enterocolitis is 11.3 keer zo hoog bij een waarde van IL-8 boven 1000 pg/mL dan wanneer die waarde onder 1000 pg/mL ligt. IL-8 in deze patiënten vormde een sterke voorspellende factor voor het beloop van de neutropene enterocolitis. Het is aan te raden in die groep patiënten vroeg te starten met intensieve ondersteunende behandeling, dus bv. in een vroeg stadium naar de Intensive Care.

In Hoofdstuk 5 wordt een 'systematische review' gepresenteerd. Zoals uit de literatuur duidelijk is en ook vanuit ons onderzoek in Zuid-Afrika blijft de centraal veneuze getunnelde lijn een risico-factor voor het ontstaan van infecties. De meeste infecties worden dan veroorzaakt door Gram-positieve organismen. Binnen de oncologie bestaat geen consensus over het gebruik van antibiotica om catheter-gerelateerde infecties te voorkomen. Dat kan bv. door het geven van antibiotica vlak voordat de catheter wordt ingebracht. Daarom is er in dit hoofdstuk gekeken naar alle gerandomiseerde studies die hierover gepubliceerd zijn tussen 1966 en 2003 in zowel volwassenen als kinder-oncologische patiënten. Alle studies die aan de selectie criteria voldeden werden vervolgens door 2 reviewers volgens kwaliteits lijsten ge-scored. Vervolgens werd een statistische analyse toegepast, waarbij gekeken werd of de studies onderling niet dusdanig verschillen dat ze niet bijelkaar genomen konden worden. Er werden 33 studies gevonden waarvan er uiteindelijk 9 geïncludeerder werden. Van deze studies rapporteerden 4 over het geven van vancomycine/teicoplanine voor het inbrengen van de lange lijn en 5 studies rapporteerden over het doorspuiten van de lange lijn met een combinatie van een antibioticum en heparine. In de 4 studies waar antibiotica voor inbrengen van de lijn gegaan werd waren er 17 patiënten met een catheter infectie (n=95) en in de controle groep(n=92) waren dit 30 patiënten. In de 5 studies waar de lijn doorgespoeld werd met de combinatie vancomycin en heparine waren er 13 patiënten met een catheter infectie (n=150) en in de controle groep (n=189) waren dit 32 patiënten. Beide methoden tonen een gunstig resultaat, dwz de statistische maat, odds ratio genoemd, toont dat het gunstig is een van beide interventies toe te passen. Om te voorkomen dat er resistentie gaat optreden tegen een van de gebruikte antibiotica is het verstandig het infectie-risico van de patiënt in ogenschouw te nemen. Bij patiënten die beginnen met de behandeling voor leukemie, bij patiënten die laag in hun afweercellen zitten en bij beenmergtransplantatie patiënten is het zeker geoorloofd een van beide interventies toe te passen.

In Hoofdstuk 6 wordt eveneens een 'systematische review' gepresenteerd waarbij het gaat over het toedienen van selectieve darm-decontaminatie (SDD) aan oncologische patiënten waarvan
verwacht wordt dat zij lage afweer cellen zullen krijgen. SDD is het geven van antibiotica oraal voordat de afweercellen laag zijn, om er voor te zorgen dat organismen in het maag-darm kanaal die je ziek kunnen maken uitgerooid worden en daardoor niet meer in de bloedbaan tot ernstige infecties kunnen leiden. Sinds de jaren 70 is deze strategie al bekend. Er is echter in de wereld geen consensus over het gebruik van SDD, dit komt vooral omdat het niet bewezen is of het gebruik van SDD er toe kan leiden dat patiënten minder aan infecties dood zullen gaan door het op tijd gebruiken van de SDD. Ook hier werden alle studies op een rij gezet tussen 1966 en oktober 2002. Er werden totaal 21 studies geïncludeerd. Hiervan waren 17 studies die SDD (Bactrimel of quinolone) vergeleken met geen SDD, en 4 studies vergeleken Bactrimel met quinolonen (zoals Ciproxin). Er werd een kwaliteitsanalyse uitgevoerd door 2 onafhankelijke artsen. Vervolgens werd de meta-analyse verricht. Uit de analyse blijkt het aantal Gram-negatieve infecties significant verminderd bij het geven van SDD. Van 59 patiënten met een Gram-negatieve infectie in de controle groep (n=517) naar 25 patiënten in de SDD groep (n=530). Er kon ook worden aangetoond dat het aantal patiënten dat overlijdt ten gevolge van een infectie verminderd met het gebruik van SDD. Van 39 patiënten die in de controle groep overlijden ten gevolge van een infectie (n=551), naar 23 patiënten in de SDD groep (n=564). Indien er een hoog risico bestaat voor infectie (meer dan 10%) dan is het zeker geoorloofd SDD voor te schrijven, welk antibioticum dan de voorkeur verdient is uit bovenstaande review niet te herleiden. Net als in het voorgaande hoofdstuk is ook hier van belang te zorgen dat er zo min mogelijk resistentie ontstaat. Daarom is het belangrijk goed te realiseren dat alleen patiënten met een kans op infectie van meer dan 10% de meeste baat zullen hebben bij het gebruik van SDD.

Hoofdstuk 7 gaat over de preventie van waterpokken, en vooral het voorkomen van de complicaties van waterpokken. Kinderen, die niet beschermd zijn tegen waterpokken en ook kanker hebben, kunnen waterpokken in een ernstige mate krijgen met een groot risico op infecties en andere ernstige complicaties. Een patiënt wordt beschreven in Hoofdstuk 7a. Dit jongetje, bekend met een leukemie, kreeg waterpokken tijdens de onderhouds-behandeling van zijn leukemie. Hij ontwikkelde daarbij een ernstige longontsteking als complicatie van waterpokken, en moest hiervoor beademd worden. De beademing verliep aanvankelijk uiterst moeizaam maar hij reageerde wel op de medicatie tegen waterpokken en herstelde langzaam maar zeker. Een manier om het waterpokken risico te verminderen in kinderen met kanker die niet beschermd zijn is kinderen te vaccineren tegen waterpokken. In sommige landen wordt dit bij gezonde kinderen al routine matig gedaan, in Nederland niet, omdat de risico’s van complicaties bij waterpokken in de gezonde populatie zo klein zijn dat er besloten is de vaccinatie niet routine matig in te voeren. Maar het is wel van belang patiënten die een hoog risico lopen op een ernstig verloop van de waterpokken te beschermen. Tot op heden zijn er veel studies verricht met levend verzwakt varicella (=waterpokken) vaccin in leukemie patiënten. In deze studies werd de chemothearpie een week voor en een week na het vaccin uitgesteld. Met deze
vaccinatie werd een goede beschermende titer gehaald, en de vaccinatie werd goed verdragen. Meest ideaal is het waterpokken vaccin vroeg in de behandeling te geven zonder uitstel van chemotherapie zodat het kind gedurende de gehele behandeling beschermd is. In Hoofdstuk 7b wordt deze studie gepresenteerd. Daarin worden voor waterpokken niet beschermde kinderen in een vroeg maar veilig stadium van hun oncologische aandoening gevaccineerd. Er werden 11 kinderen geïncludeerd. Van deze kinderen was 72.5% na een vaccinatie al beschermd. Bijwerkingen van het waterpokken vaccin waren mild. Er waren 3 kinderen met een huiduitslag die in een vroeg stadium behandeld is. Alle 3 patiënten herstelden zonder complicaties. In deze kleine groep patiënten is het bewezen dat het haalbaar is dit vaccin te geven zonder de chemotherapie te onderbreken. Deze studie zal verder uitgebreid worden.

In het afsluitende Hoofdstuk 8 worden de resultaten van de onderzoeken naar het voorspellen van de ernst van een infectie en het voorkomen van infecties verder bediscussieerd en worden voorstellen gegeven voor verder onderzoek. Er wordt gestreefd naar een nog betere overleving van onze patiënten. Door middel van uitgebreide moleculair genetische technieken worden tumoren beter begrepen, waardoor ze beter behandeld kunnen worden. Maar bij iedere behandeling blijft de “ondersteunende behandeling” van wezenlijk belang. Een belangrijk onderdeel daarvan zijn infecties die bij deze kinderen voorkomen.
Wij hopen dat dit proefschrift op een aantal vragen antwoord heeft gegeven en een stimulans zal zijn voor verder wetenschappelijk onderzoek.
Addendum
Cochrane review chapter 5
PROPHYLACTIC ANTIBIOTICS FOR PREVENTING EARLY CENTRAL VENOUS CATHETER GRAM POSITIVE INFECTIONS IN ONCOLOGY PATIENTS

van de Wetering MD, van Woensel JBM

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Date of most recent substantive amendment: 09 September 2002

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ABSTRACT

Background
Long-term tunnelled central venous catheters (TCVC) are increasingly used in oncology patients. Despite guidelines on insertion, maintenance and use, infections remain an important complication. Most infections are caused by Gram-positive bacteria. Therefore antimicrobial prevention strategies aimed at these micro-organisms could potentially decrease the majority of the TCVC infections.

Objectives
To determine the efficacy of administering antibiotics prior to insertion of a TCVC with or without vancomycin/heparin flush technique in the first 45 days after insertion of the catheter to prevent Gram-positive catheter-related infections in oncology patients.

Search Strategy
We searched MEDLINE, EMBASE, and CENTRAL up to July 2001. Reference lists from relevant articles were scanned and conference proceedings were hand searched. The authors of eligible studies were contacted to obtain additional information.

Selection Criteria
We selected randomized controlled trials giving prophylactic antibiotics prior to insertion of the TCVC, and trials using the combination of an antibiotic and heparin to flush the TCVC in oncology patients.

Data collection and analysis
Two reviewers independently assessed the studies for inclusion, extracted the data and assessed the quality.

Main Results
We included eight trials totalling 527 patients. Four reported on vancomycin/teicoplanin prior to insertion of the TCVC, and four reported on antibiotic flushing combined with heparin. The overall effect of an antibiotic prior to catheter insertion decreases the number of Gram-positive TCVC infections (odds ratio [OR] = 0.55, 95% confidence interval [CI] 0.29 to 1.04). Given an expected infection rate of TCVC during the first 45 days of up to 30% this OR implies that the number needed to treat (NNT) will be 10 (95% CI 4 to 13), this means vancomycin needs to be given to 10 patients to prevent one TCVC infection. Flushing the TCVC with antibiotics and heparin proved to be beneficial (OR = 0.35, 95% CI 0.16 to 0.77). For intraluminal colonization the baseline infection-rate is 15% which leads to a NNT of 13 (95% CI 5 to 23).

Reviewers’ conclusions
Both interventions lead to a positive overall effect but should be considered with care due to the small number of studies. Depending on the baseline TCVC infection rate it is justified to administer antibiotics prior to the TCVC insertion or to flush the catheter with a combination of an antibiotic and heparin, if the catheter-related infection rate is high.

This review should be cited as:
Patients who are treated for cancer need adequate venous access because of the frequent use of chemotherapy, requirements of intravenous fluids, blood products etc. To limit discomfort of short-term venous access long-term tunneled catheters are nowadays used in more than two thirds of paediatric and adult cancer patients (Ingram 1991; Groeger 1993). However, the use of long-term tunneled catheters is limited by the risk of blood clot formation as well as infections. This risk ranges from 1.4 (Press 1984) to 2.2 (Groeger 1993) infections per 1000 catheter days. About one third of patients experience an episode of infection while having the TCVC in place. The organisms cultured are Gram-positive organisms (70%), Gram-negative organisms (15%) fungal organisms (8%) and anaerobic organisms (7%).

The adherence to and colonization of TCVC with micro-organisms is facilitated by the formation of a very thin biofilm inside the catheter lumen. This process is influenced by several factors such as the production of fibroglycocalyx (extracellular slime) by coagulase negative staphylococci. In addition, the host reaction to the TCVC results in the formation of a thrombin sleeve rich in clotting factors such as fibronectin, fibrinogen, and fibrin which contributes to the formation of the biofilm (Darouiche 1999). This means that adequate antibiotic treatment may lead to resolution of the TCVC infection only in certain cases (i.e. when caused by coagulase negative staphylococci) whereas in other cases (i.e. when caused by pseudomonas, staphylococcus aureus, or fungi) this will be much more difficult to clear and therefore removal of the catheter is necessary.

The organisms responsible for catheter colonization and infection come from four sources: the skin, the catheter hub (the part through which the catheter is tunneled under the skin), haematogenous seeding (infections originating outside the catheter can reach the TCVC via the bloodstream) and contamination of the intravenous fluids given to the patient (for instance intravenous total parenteral nutrition) (Farr 1995).

Early catheter infections (infections that develop within 45 days after placement of the catheter) are mostly due to organisms from the skin insertion site. After 45 days the catheter hub becomes a far more important source of infection (Shaul 1998). Because of the increased chance of early catheter infections within days of placement, internal guidelines were developed to prevent these infections, such as:

- a) aseptic technique of insertion of the catheter,
- b) protocols for care and handling of the catheter,
- c) adequate information to all who handle the catheter,
- d) restriction on the number of interruptions of the catheter (the number of times per day one is allowed to open the catheter, to give medication or to draw blood).

Despite international guidelines that have been developed by Hospital Infection Control Practices Advisory committee (CPAC 1990) about 15-20% early TCVC infections are still seen. It has been shown (Lim 1993) that the incidence of Gram-positive infections is increasing. The increase is probably due to two factors:

1) an increase in the use of central venous catheters (mainly Hickman/Broviac catheters),
2) the use of high-dose chemotherapy, which destroys the normal mucosal protective barriers of the upper respiratory tract and the gastrointestinal tract.

Therefore reduction of Gram-positive infections by introducing antibiotics in an early stage may be effective to decrease specific catheter-related bloodstream infections. Antibiotics can be introduced in two ways, either prior to the insertion of the catheter, or by flushing the catheter with a combination of an antibiotic and heparin during the life-span of the catheter. It is known that the risk of infections is lower in internal cvc's (ports) than in external devices (Broviac or Hickman). However, it has been shown that this difference only becomes significant after more than one year of catheter use and therefore data on different tunneled cvc types were pooled in this analysis. The risk of infection is greatest during the first 100 days after placement (Salzman 1995; Wurzel 1988).

This systematic review assesses the effectiveness of prophylactic antibiotics in the prevention of early Gram-positive catheter related infections, when the risk of infections is greatest. The importance of the first 45 days allows us to cover the induction period of chemotherapy. This is the time-period that many manipulations of the TCVC are necessary because of the intensity of the chemotherapy. For this reason both internal and external tunneled central venous catheters could be included in the review.

In reviewing the papers both adults and children were included, as the adult patient is comparable to the paediatric patient as far as interpreting infections in central venous catheters.
OBJECTIVES

To determine the efficacy of administering antibiotics prior to insertion of a tunneled central venous catheter with or without vancomycin/heparin flush technique in the first 45 days after insertion of the catheter to prevent Gram-positive catheter-related infections in cancer patients.

CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

Types of studies

- Randomized controlled trials (RCTs) comparing placebo versus antibiotics prior to insertion of the catheter to reduce Gram-positive infections related to the catheter
- RCTs comparing the heparin flush technique versus the antibiotic/heparin flush technique to reduce Gram-positive infections due to catheter related infections
- RCTs combining the two interventions

Types of participants

Patients with cancer (both adults and children) who will have a tunneled central venous catheter inserted and are likely to receive chemotherapy causing episodes of neutropenia, with increased risk of infection.

Types of intervention

- Drug interventions
- Administering antibiotics prior to catheter insertion
- Adding an antibiotic to the normal heparin flush technique

Types of outcome measures

The major outcome indicator is bacteraemia due to central venous catheter infections. Proxy measures are the frequency of exit-site infections and tunnel-infections. The time frame for assessment of outcomes within a study should be stated, (establishing when the catheter related infection occurred from the time of insertion).

Definitions (Mermel 2001):

Infections related to the catheter should be defined as follows:
*exit-site infections: 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.
*tunnel infection: This involves spreading cellulitis overlying the tunnel tract of subcutaneously tunnelled-catheters. There are signs of inflammation along the tunnel tract and there is tenderness to palpation over the tunnel tract.

Definite catheter infection:
* 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.

*Catheter-related infection can also be defined when there is a temporal succession of catheter flushing, onset of chills and fever and a positive blood culture, then this is highly suggestive of a catheter-related infection.

In addition, 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.

SEARCH STRATEGY FOR IDENTIFICATION OF STUDIES

See: Cochrane Gynaecological Cancer Group search strategy

MEDLINE and EMBASE search was done for the years 1966-2000 and a search of CENTRAL on the Cochrane Library (Issue 4, 2000) was done.

Search terms: (Broviac OR Port-a-cath OR Hickman OR exp catheterization,central venous). AND ( prophylactic antibiotics OR vancomycin OR teicoplanin) AND (exp oncolog? OR cancer OR malignancy OR neoplasm) AND
Identification of studies meeting the eligibility criteria was performed independently by two reviewers (MvdW, JvW).

Decisions on which trials to include were based on the methods section of the trial. Data-extraction included, the patient group involved, the intervention described, the outcome assessed, and the analysis done.

The methodological quality was initially assessed using the Jadad criteria, (blinding of randomization, inclusion and exclusion criteria, intervention performed, and outcome looked at), thereafter scored using the Tuldø criteria (Tuldø 1997), including assessment of the quality of randomization, blinding and analysis. Authors were contacted for additional information where necessary. Disagreements were resolved by discussion between the reviewers.

Allocation concealment was assessed using the scale set out in the Cochrane Collaboration Handbook for Reviewers (Cochrane Handbook).

Grade A) Adequate: Some form of centralised or pharmacy controlled randomization scheme, or the use of pre-coded identical containers administered sequentially to patients or the use of sequentially numbered sealed opaque envelopes. Alternatively using an on site computer with a locked file which could only be accessed after entering participant details or a mixture of these approaches, including innovative schemes, provided that the method appears impervious to allocation bias.

Grade B) Uncertain: When only terms such as lists or tables or sealed envelopes or randomly assigned were mentioned in the text, or any trial where intervention or placebo assignments were mentioned without specifying the method of allocation.

Grade C) Inadequate: When alternation, date of birth, case record, day of the week, enrolment order etc were used, or when an open system of random numbers or assignment were used.

The studies were divided into two groups which were analysed separately.

1: prophylaxis with antibiotics at insertion of the central venous catheter versus no prophylaxis.
2: vancomycin/heparin flush technique versus only heparin flush technique.

Statistics:
The outcomes were weighted by inverse variance. Besides the fixed effect model the random effect model was used to calculate the effects because the included studies were heterogeneous in design, intervention and study population. The number of catheter related infections was used as the primary endpoint. Results are presented with 95% confidence intervals (CIs).

DESCRIPTION OF STUDIES

We identified 11 studies. Three studies were excluded. One study was excluded because it involved only neonates (Ocete 1998) and two studies were excluded because non-tunneled catheters were used (Raad 1998; Carratala 1999).

Of the eight included studies, four addressed the administration of antibiotics prior to insertion of the catheter (Vassiliomaniakis 1996; Lim 1993; Ranson 1990, Ljungman 1997), and four studies addressed flushing the catheter with the combination of vancomycin and heparin (Rackoff 1995; Schwartz 1990; Barriga 1997; Henrickson 2000). The total number of patients in the eight included studies was 527, four studies were done in adults (n=252), three studies in children (n=192), and one trial combined children and adults (n=83). No extra information was obtained from the conference proceedings. In Table 4 all abstracts on central venous catheters and infection rates have been summarized to give information on the rates of infection in the different units. This may be important in interpreting the results of the included studies.
METHODOLOGICAL QUALITY

In six studies the method of blinding was adequately described and two studies were not adequately blind.
Initially in one study randomization was performed but later all patients were included in the experimental group.
In our analysis we only used the first part of the study when randomization was performed (Vassilomaniakis 1995). In the second study an open randomization was performed and the study was stopped at interim analysis (Ljungman 1997). Due to the poor internal validity of the latter study in comparison to the three other studies included two analyses are presented.

Eligibility criteria
All studies described the eligibility criteria sufficiently. All the studies excluded patients already receiving vancomycin, or other systemic antibiotics and selective gut decontamination use was allowed (that is the use of oral antibiotics starting before a neutropenic episode is expected in which the potentially pathogenic aerobic organisms are eliminated without affecting the non-pathogenic anaerobic organisms). One study only included patients not neutropenic at the start of the study. The others did not specify this aspect at the start of the study but specified that these patients would become neutropenic due to their disease. All paediatric studies specified children to be less than 20 years of age. All studies specified the catheter used (in all studies a Hickman catheter was used, double or single-lumen)

Treatment allocation:
In five out of eight studies the treatment allocation was concealed (Grade A as described in the methodological quality of the studies). Two studies used a quasi-randomization method (Grade B, Vassilomaniakis 1995; Lim 1993), and one study did not specify the method of randomization (Ljungman 1997) In all studies the index intervention and control intervention was explicitly described. In three out of eight trials the participants were not blinded to the treatment (Vassilomaniakis 1995; Lim 1993; Ljungman 1997). In four out of eight trials the outcome assessor was not blinded to the intervention (Vassilomaniakis 1995; Ranson 1990; Lim 1993; Ljungman 1997). In two out of eight trials the outcome measures were not clearly stated (Vassilomaniakis 1995; Ranson 1990).

See additional tables for the criteria list for the assessment of the methodological quality of included studies (Tulder 1997); Table 01 gives these criteria, Table 02 the internal validity scores and Table 03 the external validity scores.

RESULTS

Comparison 1: The four studies evaluating antibiotic prophylaxis before insertion of the central venous catheter were pooled (Vassilomaniakis 1995; Lim 1993; Ranson 1990; Ljungman 1997). In two studies teicoplanin and not vancomycin was used as the intervention before insertion of the catheter (Lim 1993; Ljungman 1997). Since we aimed to review the prevention of Gram-positive TCV C infections and both vancomycin and teicoplanin are glycopeptides, that are active against Gram-positive bacteria we felt it was felt acceptable to pool these data. The odds ratio under a fixed effect model was 0.55 (95% CI 0.28 to 1.04). Because of heterogeneity the random effect model was applied resulting in an OR of 0.54 (95% CI 0.17 to 1.74).

To interpret the results knowledge of the baseline infection rate of the TCV C is important. With this knowledge one can derive the number needed to treat from the odds ratio (Cochrane Handbook (appendix 8b)) If the catheter related infection rate approaches 15% an OR of 0.55 will give a NNT of 18 (this means vancomycin should be given to 18 patients to avoid one catheter related infection). If the catheter related infection rate is 30% vancomycin should be given to 10 patients to prevent one catheter related infection. The overall effect favours treatment with an antibiotic prior to insertion of the catheter, although the catheter related infection rate is of importance. Excluding the study of Ljungman because of poor internal validity, the OR under a fixed effect model was 0.46 (95% CI 0.24 to 0.91). The NNT will then range from eight to 20 patients.

Comparison 2: The four studies using vanco/heparin flush method compared to heparin only flush (control) were pooled (Schwartz 1990; Raekoff 1995; Barriga 1997; Henrickson 2000). There was no heterogeneity in the patient groups included. All were oncology patients who needed chemotherapy, mainly children.
Statistical results showed using the fixed effect model an OR of 0.35 (95% CI 0.18 to 0.77). Using the random effect model the OR was 0.39 (95% CI 0.16 to 0.95). If the risk of a catheter related sepsis is 10% then the NNT to prevent one catheter related infection will be 17, if this risk is 20% then the NNT is nine. The overall effect favours treatment with an antibiotic in the flush solution to prevent catheter related infections, although the catheter-related infection rate is of importance.
DISCUSSION

1: To assess the effect of antibiotics before insertion of the catheter.

Study design and methodology of the four included studies were sufficient to pool and analyze the data. The study of Vassilomanaki in 1995 was the most difficult to interpret because of the initial randomization followed by treating all participants with antibiotics before insertion of the catheter. The combined overall EER (experimental event rate) and the CER (control event rate) were 14.8% and 24.6% respectively. Depending on the background tunnelled catheter related infection rate that ranges from 10% to 30%, the NNT will range from 10 to 18 patients.

If the risk of infection is high (for instance neutropenic patients and/or patients on induction therapy for haematological malignancies (cancer of the blood) one should consider giving antibiotics prior to insertion of the catheter. In this group of patients the skin insertion site is the source of introducing vancomycin-sensitive organisms (VCO). Prevention of infection at this level will decrease morbidity in these patients.

2: To assess the effect of antibiotic flushing of the catheter.

Design and methodology of the four included studies were sufficient to pool and analyze the data. Two studies (Schwartz 1990; Rackoff 1995) had relatively small groups of patients. Henrickson 2000 and Barriga 1997 included larger groups of patients. The combined overall EER (experimental event rate) and the CER (control event rate) were 8.6% and 18.8% respectively. Barriga 1997 pointed out that intraluminal colonization of central venous catheters with microorganisms probably occurs mainly during non-neutropenic episodes, whereas during neutropenia other sources of infection play a major role, like the gastro-intestinal tract. Depending on the incidence of TCRS (ranging from 5 to 15%) the NNT ranges from 13 to 23 patients.

In both cases vancomycin/telcoplanin prior to insertion of the central venous catheter and vancomycin/heparin flushing of the central venous catheter may develop the problem of bacterial resistance to vancomycin. With the doses flushed this seems unlikely as the dose is so small it does not distribute systemically (25 microgram/ml using 3 mls). As for the antibiotic prior to insertion of the catheter, this involves only one or two doses, and if qualifying the definition that only certain patients will receive this treatment it does not imply a danger of developing resistance.

REVIEWER’S CONCLUSIONS

Implications for practice

Both interventions were effective in preventing Gram-positive tunnelled catheter-related infections but the analyses should be considered with care due to the small number of studies. If the tunnelled central venous catheter infection rate is expected to be high, then it can be justified to use antibiotics prior to insertion of the central venous catheter, or to flush the central venous catheter with a combination of an antibiotic and heparin.

Implications for research

As indicated it would be useful to know in which risk groups one would implement the interventions. Although some of the included studies stratified risk groups, none analyzed these separately because of small numbers. Therefore a multicentre RCT answering the question of whether the above interventions are justified in the specific risk groups is of extreme importance. The future direction could be coating of longterm tunnelled central venous catheters with antibiotics compared to the above interventions to decrease Gram-positive catheter-related infections.

ACKNOWLEDGEMENTS

We wish to thank our funders for the research grant which made this systematic review possible. We would also like to thank Dr R. Scholten, epidemiologist at the AMC for reviewing the manuscript and assisting with the statistical part.

POTENTIAL CONFLICT OF INTEREST

None known
### TABLES

#### Characteristics of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>Participants</th>
<th>Interventions</th>
<th>Outcomes</th>
<th>Notes</th>
<th>Allocation concealment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barriga 1997</td>
<td>double blind randomization</td>
<td>n=83 adult and paediatric patients various malignancies, mainly leukaemia, 143 febrile episodes recorded</td>
<td>vanco vs heparin flush (25 ug/ml vanco and 25 units/ml hepar)</td>
<td><em>bacteraemia</em> <em>Vancomycin-sensitive organism bacteraemia</em></td>
<td>a difference was stated in neutropenia and nonneutropenia</td>
<td>A</td>
</tr>
<tr>
<td>Henrikskon 2000</td>
<td>double blind randomization stratified for riskgroups</td>
<td>n=128 paediatric patients (44% ALL, 40% solid, 7% BMT) There were 153 assessable TCVC</td>
<td>vanco versus heparin flush (25 ug/ml vanco and 100 units/ml hepar)</td>
<td><em>exit-site infection</em> <em>bacteraemia</em> <em>time to first infection</em></td>
<td>the third group included vanco-heparin ciprofloxacin</td>
<td>A</td>
</tr>
<tr>
<td>Lim 1993</td>
<td>method of randomization not clear</td>
<td>n=88 adult oncology patients, haematological malignancies</td>
<td>teicoplanin before insertion 400 mg before insertion catheter vs control</td>
<td><em>soft-tissue infection</em> <em>catheter related sepsis</em></td>
<td>all episodes of CRS occurred in patients who were neutropenic</td>
<td>B</td>
</tr>
<tr>
<td>Ljungman 1997</td>
<td>method of randomization not clear</td>
<td>n=66 adult oncology patients, BMT and leukemia patients</td>
<td>teicoplanin prior to insertion and 24 hrs after insertion</td>
<td><em>bacteraemia</em> <em>exit-site infection</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes</td>
<td>At interim analysis the preset efficacy could not be met therefore study stopped</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allocation concealment</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Rackoff 1995</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methods</td>
<td>double blind randomization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants</td>
<td>n=55 paediatric patients, one centre (total group was 63 patients, 8 were receiving TPN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>Analysis was done on the oncology patients only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcomes</td>
<td>vancomycin versus heparin flush (25 ug/ml vanco and 100 units/ml heparin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes</td>
<td>*bacteraemia with a vanco sensitive organism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allocation concealment</td>
<td>*time to first infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Study                                      | Ranson 1990                                                                      |
| Methods                                    | double blind randomization                                                       |
| Participants                               | n=98 adult patients                                                             |
| Intervention                               | A) N=48 and 35 catheters, acute leukemia and BMT                                  |
| Outcomes                                   | B) N= 50 and 37 catheters (solid tumor)                                          |
| Notes                                      | vancomycin vs control (2 doses one prior to insertion, one after positioning of the catheter 500 mg vanco) |
| Allocation concealment                     | *catheter related sepsis in first 30 days                                        |
|                                           | *tunnel sepsis                                                                   |
|                                           | *CNS bacteraemia                                                                |

| Study                                      | Schwartz 1990                                                                   |
| Methods                                    | Double blind randomization                                                       |
| Participants                               | n=45 paediatric patients                                                        |
| Intervention                               | vancomycin versus heparin flush(25 ug/ml vanco and 100 units/m)                  |
| Outcomes                                   | *bacteraemia                                                                     |
|                                           | (quantitative culture)                                                           |
|                                           | *time to first inf.                                                              |
| Notes                                      | statistics on the number of children not catheters                               |
|                                           | quantitative blood cultures                                                      |
| Allocation concealment                     | A                                                                                |

| Study                                      | Vassilomanakis 1995                                                              |
| Methods                                    | Randomization by cards in closed envelopes,                                     |
| Participants                               | n=40 adult patients                                                             |
| Intervention                               | vancomycin vs control (vanco in 3 doses of 500 mg 1 hr prior to insertion, 6 and 12 hr afterwards) |
| Outcomes                                   | *exit-site infection                                                            |
|                                           | *cvc related bacteraemia                                                         |
|                                           | *Gram+infections                                                                |
Notes: only initially randomized

Allocation: B

Concealment

Vs = versus
CRS = Catheter related sepsis
BMT = Bone marrow transplant
TCVC = Tunneled central venous catheter
TPN = total parenteral nutrition
CVC = central venous catheter
vanco = vancomycin
heparin
ALL = acute lymphoblastic leukaemia
mg = milligram(s)
ug = microgram(s)
ml = millilitre(s)
n = number of participants

Characteristics of excluded studies

Carratana 1999

Adult haematology patients with non-tunneled CVC's received 10U heparin per ml (n=57) or 10U heparin+25 ug. vancomycin per ml (n=60) allowed to dwell in catheter 1 hour every 2 days. Catheter-related bacteraemia in 7% of patients in control group, and 0% in experimental group (p=0.05). Mainly excluded because non-tunneled catheters.

Ooste 1998

Single centre trial 2 groups. Control group - 61 newborns, experimental group 85 newborns, all receiving a central catheter (umbilical artery, umbilical vein and/or silastic). The study group received prophylactic vancomycin 25 ug/ml. All patients received parenteral nutrition. Results CNS 21/61 in the control group and 19/85 in the vancomycin group (p<0.05). The patient group is not the group studied in this review. Methods of the study poor. Not specified how often the prophylactic vanco was given. Clinical criteria were used to determine if the neonate was infected, then peripheral and central cultures were done. Not specified if quantitative or qualitative cultures were done. Trial not blinded, no tunneled catheters used, no appropriate patient group.

Raad 1998

Crossover study: 26 patients with melanoma on IL2 treatment enrolled. All patients received a double lumen non-tunneled silicone catheter in subclavian vein. Patients randomized to receive prophylactic antibiotics novobiocin 500 mg + rifampin 300 mg orally. Significant results 41% in control group catheter related bacteraemia and 8% in experimental group, excluded because of non-tunneled catheters. Very specific group with high incidence of infection, not representing the group aimed at in this Cochrane review.

ADDITIONAL TABLES

Table 01 Criteria list for the assessment of methodological quality of included studies

<table>
<thead>
<tr>
<th>Item ID</th>
<th>Description</th>
<th>Implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient</td>
<td>Note: all criteria were scored yes(+), no(-) or don't know</td>
<td></td>
</tr>
<tr>
<td>selection</td>
<td>Were the eligibility criteria specified?</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Patient inclusion/exclusion criteria must have been described appropriately according to the reviewer</td>
<td></td>
</tr>
</tbody>
</table>
A random (unpredictable) allocation must have been applied
Allocation should have been performed by an independent
person not responsible for determining eligibility for inclusion.
groups must be similar at baseline with regard to at least three
of the four prognostic indicators of age sex duration of
symptoms and value of main outcome measures

adequate description of the experimental intervention so that
treatment can be replicated
adequate description of the control intervention so that
treatment can be replicated
co-interventions should either have been avoided in the trial
design or be similar in the 2 groups
adequate information about blinding must have been provided

Adequate information about blinding must have been provided
at least one of the following outcome measures must be
included: catheter related sepsis, exit infections, tunnel
infections and time to first infection
any adverse events should be noted
included patients who did not complete the follow up period or
were not included in the analysis should be described, if the
percentage of drop outs is less than 20% then a '+' is scored
outcome assessment after randomization
Timing of outcome assessment should have started from the
moment of treatment allocation and be identical for all
intervention groups and all important outcome measures

sample size should have been presented for each group at
randomization and for the most important outcome measures
for all randomized patients the most important moments of
effect measurement should have been reported
For continuous data mean, median, standard deviation with 95
% confidence interval should be presented. For nominal and
ordinal outcomes the number of patients to whom the outcome
measure applies and the total number of patients must be
presented.

Table 02 internal validity scores(b1,b2,c,e,f,g,j,l,n)

<table>
<thead>
<tr>
<th>reference</th>
<th>b1</th>
<th>b2</th>
<th>c</th>
<th>a</th>
<th>f</th>
<th>g</th>
<th>j</th>
<th>l</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vassilomaniakis 1995</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 03 External Validity (a, d1, d2, h, l, k, m, o)

<table>
<thead>
<tr>
<th>Reference</th>
<th>a</th>
<th>d1</th>
<th>d2</th>
<th>h</th>
<th>l</th>
<th>k</th>
<th>m</th>
<th>o</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vassilomaniakis 1995</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Ranson 1990</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lim 1993</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Barriga 1997</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Rackoff 1995</td>
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<td>Schwartz 1990</td>
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</tr>
<tr>
<td>Henrickson 2000</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ljungman 1997</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1997: O-54: Retrospective, 149 ports in 135 patients 8% infected, CNS majority of infections.

1997: P-227: Retrospective, 51 catheters in 37 patients. Clinically significant infection 12.2 per 1000 catheter days (CNS)

1997: O-65: Prospective randomized trial. Teicoplanin induction ALL 25 patients, 25 patients no teicoplanin, no difference in gram-positive organisms

1997: P-259: Retrospective study 355 children. Infectious

Table 04 Abstracts congresses

SIOP

1995 J1: prospective 30 adults CVC inserted CNS isolated from 5 out of 30 catheter tips.

1995 J2: retrospective. 984 catheters in 837 patients. Infection 12.7% .1.26 per 1000 catheter days.

1995 J10: 151 patients were randomized to new hub connector n=78 or standard connector n=73 CNS infection exp arm 4% control arm 16%

ICAAC

1995: J1723: Risk of sepsis associated with CVC in children with leukemia. The relative risk of fever and neutropenia is increased OR=1.3 (1.00-1.69)

1995: J1791: Complications of CVC. The rate of catheter related infections was 15.8% (95% CI 11.6-20.6%) Most infections in leukemic patients who were neutropenic at time of insertion.

ASCO

1996 129: retrospective: In 101 patients 97 single lumen and 7 double lumen access ports implanted. 6 cases of infection.

1995 1723: Risk of sepsis associated with CVC in children with leukemia. The relative risk of fever and neutropenia is increased OR=1.3 (1.00-1.69)

1995 1791: Complications of CVC. The rate of catheter related infections was 15.8% (95% CI 11.6-20.6%) Most infections in leukemic patients who were neutropenic at time of insertion.

1996 56: descriptive: skin and catheter hub major sources of infection. CNS can be treated without catheter removal. Staph. aureus complications resulted in catheter removal.

1999 0-35: retrospective. 23 patients positive Staph. aureus. 22 had CVD, 195 patients CNS positive, 135 had a CVD. Study meant to give insight into distribution of Staphylococcal infections.

1999 P-94: Effect of Tauriform in treatment of device related bacteraemia a pilot trial. 8 patients with a device associated
complications 2.8 per 1000 catheter days. CRS in 10 cases, majority CNS infections devices were removed. 7 bacteraemia had Tauroline installed. Clinical signs of bacteraemia disappeared, blood cultures negative

1998 U-98: retrospective 502 catheters in 388 pts peroperative complications in leukemia 2.5% and in solid tumours 0.8%
1996 J-103: continuous low dose vanco to prevent gram positive infections. Low dose vanco (2.5 mg/100 ml) in TPN control group: 29 per 100 infants exp group: 5 per 100 infants CNS infection
2000 P-313: retrospective 32 catheters CRS 8.2 per 1000 catheterdays, in 55%
CNS.

REFERENCES

References to studies included in this review

Barriga 1997 (published data only)

Henricksson 2000 (published data only)

Lim 1993 (published data only)

Ljungman 1997 (published data only)

Rackoff 1995 (published data only)

Ranson 1990 (published data only)

Schwartz 1990 (published data only)

Vassilomaniakis 1995 (published data only)

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References to studies excluded from this review

Carratala 1999

Ocete 1998

Raad 1998

Additional references
Cochrane Handbook

CPAC 1990

Darouiche 1999

Farr 1995

Groeger 1993

Ingram 1991

Merme 2001

Press 1984

Salzman 1995

Shaull 1998

Tuulder 1997

Wurzel 1988
### COVER SHEET

**Title**

Prophylactic antibiotics for preventing early central venous catheter Gram positive infections in oncology patients

**Reviewer(s)**

van de Wetering MD, van Woensel JBM

**Contribution of reviewer(s)**

- **Contact reviewer:** M.D. van de Wetering - reference search, article retrieval, assessment of studies for inclusion/exclusion, data-extraction analysis, manuscript preparation.
- **Co-reviewer:** J. van Woensel - Reference search, assessment of studies for inclusion/exclusion, data extraction, reviewing of manuscript.

**Issue protocol first published**

2001/4

**Issue review first published**

2003/2

**Date of most recent amendment**

31 July 2001

---

### TABLE

#### 01 vancomycin versus control

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 catheter related sepsis</td>
<td>4</td>
<td>252</td>
<td>Odds Ratio (Fixed) 95% CI</td>
<td>0.55 [0.29, 1.04]</td>
</tr>
</tbody>
</table>

#### 02 vancomycin flush versus heparin flush

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 catheter related sepsis</td>
<td>4</td>
<td>275</td>
<td>Odds Ratio (Fixed) 95% CI</td>
<td>0.35 [0.16, 0.77]</td>
</tr>
</tbody>
</table>

#### 03 vancomycin/teicoplanin versus control

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 analysis of vancomycin/teicoplanin prior to insertion catheter excluding Llunmgman study, outcome CRS</td>
<td>3</td>
<td>187</td>
<td>Odds Ratio (Fixed) 95% CI</td>
<td>0.46 [0.24, 0.91]</td>
</tr>
</tbody>
</table>
PROPHYLACTIC ANTIBIOTICS OR CATHETER FLUSHING WITH VANCOMYCIN AND HEPARIN MAY HELP CANCER PATIENTS AT HIGH RISK OF CATHETER-RELATED INFECTIONS

Patients with cancer often need to be given drugs and other treatments intravenously, so are frequently fitted with long-term tunneled catheters. Infections sometimes occur. Evidence from randomised controlled trials shows it
may be useful to give prophylactic antibiotics prior to catheter insertion or to flush the catheter with combined vancomycin and heparin, but microbial resistance may occur unless this practice is limited to high-risk patients.

Index Terms

Medical Subject Headings (MeSH)
Antibiotic Prophylaxis; Catheterization, Central Venous [adverse effects]; Gram-Positive Bacterial Infections [prevention & control]; Neoplasms [therapy]; Randomized Controlled Trials

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**GRAPHS**

Review: Prophylactic antibiotics for preventing early central venous catheter Gram-positive infections in oncology patients
Comparison: 01 vancomycin versus control
Outcome: 02 catheter related sepsis

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>Odds Ratio (Fixed) 95% CI</th>
<th>Weight (%)</th>
<th>Odds Ratio (Fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lim 1993</td>
<td>7/43</td>
<td>16/46</td>
<td></td>
<td>60.0</td>
<td>0.35 [0.13, 0.97]</td>
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<td>Youngman 1997</td>
<td>2/33</td>
<td>0/32</td>
<td></td>
<td>1.0</td>
<td>5.10 [0.24, 111.77]</td>
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<tr>
<td>Ranson 1980</td>
<td>9/38</td>
<td>9/38</td>
<td></td>
<td>26.1</td>
<td>1.00 [0.34, 2.91]</td>
</tr>
<tr>
<td>Vassilomanakis 1995</td>
<td>1/16</td>
<td>5/11</td>
<td></td>
<td>21.5</td>
<td>0.08 [0.01, 0.84]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>10/128</td>
<td>30/124</td>
<td></td>
<td>100.0</td>
<td>0.55 [0.20, 1.84]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: chi-square=6.57 df=3 p=0.0898
Test for overall effect:-1.85 p<0.00
Review: Prophylactic antibiotics for preventing early central venous catheter Gram positive infections in oncology patients
Comparison: 02 vancomycin flush versus heparin flush
Outcome: 01 catheter related sepsis

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>Odds Ratio (Fixed)</th>
<th>Weight</th>
<th>Odds Ratio (Fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>95% CI</td>
<td></td>
<td>95% CI</td>
</tr>
<tr>
<td>Barriga 1997</td>
<td>7/36</td>
<td>10/44</td>
<td>0.36 [0.14, 1.00]</td>
<td>92.6</td>
<td>0.36 [0.14, 1.00]</td>
</tr>
<tr>
<td>Henriksson 2000</td>
<td>1/28</td>
<td>7/64</td>
<td>0.30 [0.04, 2.58]</td>
<td>17.0</td>
<td>0.30 [0.04, 2.58]</td>
</tr>
<tr>
<td>Rackoff 1995</td>
<td>2/24</td>
<td>1/27</td>
<td>2.00 [0.17, 23.44]</td>
<td>4.1</td>
<td>2.00 [0.17, 23.44]</td>
</tr>
<tr>
<td>Schwartz 1990</td>
<td>0/21</td>
<td>8/24</td>
<td>0.07 [0.00, 1.28]</td>
<td>25.6</td>
<td>0.07 [0.00, 1.28]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>10/118</td>
<td>30/150</td>
<td></td>
<td>100.0</td>
<td>0.35 [0.16, 0.77]</td>
</tr>
</tbody>
</table>

Test for heterogeneity chi-square=3.19 df=3 p=0.3528
Test for overall effect=-2.61 p=0.11

Favours treatment  Favour s control

Review: Prophylactic antibiotics for preventing early central venous catheter Gram positive infections in oncology patients
Comparison: 03 vancomycin/rebeclaparin versus control
Outcome: 01 analysis of vancomycin/rebeclaparin prior to insertion catheter excluding Lungman study, outcome CRS

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>Odds Ratio (Fixed)</th>
<th>Weight</th>
<th>Odds Ratio (Fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>95% CI</td>
<td></td>
<td>95% CI</td>
</tr>
<tr>
<td>Lim 1993</td>
<td>7/43</td>
<td>18/46</td>
<td>0.35 [0.13, 0.97]</td>
<td>51.5</td>
<td>0.35 [0.13, 0.97]</td>
</tr>
<tr>
<td>Ranso 1990</td>
<td>9/30</td>
<td>9/30</td>
<td>1.00 [0.34, 2.61]</td>
<td>26.0</td>
<td>1.00 [0.34, 2.61]</td>
</tr>
<tr>
<td>Vassilomanolakis 1995</td>
<td>1/10</td>
<td>5/11</td>
<td>0.06 [0.01, 0.84]</td>
<td>21.9</td>
<td>0.06 [0.01, 0.84]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>17/95</td>
<td>30/92</td>
<td></td>
<td>100.0</td>
<td>0.40 [0.24, 0.67]</td>
</tr>
</tbody>
</table>

Test for heterogeneity chi-square=4.43 df=2 p=0.1093
Test for overall effect=-2.23 p=0.03

Favours treatment  Favour s control
Publications
Publications


- van de Wetering M.D, Poole J, Friedland I, Caron H.N. Bacteraemia in a paediatric oncology unit in South Africa. *Medical and Paediatric Oncology* 37: 525-531, 2001


Book Chapters


Abstracts

1 van de Wetering M.D. Poole J, Friedland I. Bacteremia in a pediatric South African oncology unit: results of a retrospective study. 
*Poster presentation: SIOP(Societe internationale d’oncologie pediatrique) 1995 Montevideo*

2 van de Wetering M.D. de Tollenaer S, Vossen J, Friedland I, Poole J. Bacteremia in pediatric oncology patients: a Retrospective study comparing two university hospitals. 
*Poster presentation: ESPID (European society of paediatric infectious diseases)1997 Paris (France)*

*Poster presentation SIOP Amsterdam 2000*


5 van de Wetering M.D. Prevention and treatment of infectious complications in paediatric oncology patients. *Oral presentation Dutch paediatrics association(NVK) meeting 2000, Veldhoven(NL)*

*Oral presentation SIOP Brisbane October 2001* 
Presented as "masterclass" 8-2-02 AMC/EKZ , Amsterdam

*Poster presentation ASH, Abstract in Blood, 98, 473, 2001 (Abstract American Society Haematology)*

*Poster presentation: MASCC (multinational association supportive care in cancer)Boston(USA) June 2002*

*Oral presentation SIOP Cairo, 2003* 
Awarded with the IPSO award 2003

*Poster presentation SIOP, Cairo 2003*
Siyaborga

dankwoord
En dan eindelijk het dankwoord....
Heel lang heb ik er naar uitgezien dit te schrijven, want het betekent dat het boekje klaar is, en ik dacht dat dat moment nooit zou komen.......

Allereerst wil ik alle patientjes bedanken, voor het meewerken aan alle onderzoeken. Zonder jullie was dit proefschrift niet tot stand gekomen. Een patientje vroeg mij op de poli “sta ik nu in je boekje”, word ik nu beroemd ? Nee, namen staan niet in het boekje, maar voor mij zijn jullie veel beroemder dan alle beroemdheden samen, jullie zijn stuk voor stuk “kanjers” en ik hoop dat al het onderzoek in dit boekje ertoe bij draagt dat “toekomstige patientjes” minder last hebben van infecties en minder infecties zullen krijgen, en daar hebben jullie allemaal je steentje aan bijgedragen.

Prof Dr Voûte, beste Tom, door jou heb ik de stap gezet om een proefschrift te beginnen, je enthousiasme en nieuwe ideeën stimuleren zo, dat je een echte klinicus toch zover krijgt om een proefschrift te schrijven. Al tijdens mijn kindergeneeskunde opleiding in Johannesburg had je ideeën over de relatie voeding en kanker, maar voor onderzoek was in Baragwanath hospital niet veel tijd en was ik al lang blij dat ik voor vertrek naar Nederland mijn mastersproject kon afronden. Het stokje van promotor schap heb je overgegeven aan Huib Caron, maar ik ben wel heel blij dat je in de promotiecommissie zit, dank voor je vertrouwen in mij.

Prof Dr Caron, beste Huib, toen Tom het stokje van promotor aan jou doorgaf, moest ik daar in het begin erg aan wennen. Jij bent zo slim, zo gedreven, eist zoveel, ik dacht dat kan ik nooit bijbenen. Er is in het begin dan ook menig traan gevallen. De uitspraak “delete na de introductie” zal ik niet gauw vergeten. Maar Huib zonder jou input, ideeën en discussies over wat de beste manier was om het artikel op te zetten, wat de kern van het onderzoek is en hoe je het over wil brengen op anderen zou ik dit werk nooit op deze manier voltooid hebben, bovendien maakte je altijd tijd vrij in je drukke programma, en las je de zoveelste versie binnen een week zodat ik weer verder kon. Ik heb dat heel erg gewaardeerd en had mij geen betere promotor kunnen wensen. Je hebt me zelfs zover dat ik ook enthousiast ben geworden voor vervolg projecten. Duizendmaal dank...

Prof Dr Kuijpers, beste Taco. Een aanzienlijk deel van het proefschrift combineert de oncologie met de immunologie dus was het vrij logisch dat jij de copromotor werd, en daar was ik heel dankbaar voor. Hoe druk je ook was je vond altijd tijd een zoveelste versie snel voor me na te kijken. Als er weer een presentatie moest worden gehouden over neutropene enterocolitis dan zorgde je ervoor dat de powerpoint slides er perfekt uitzagen en dat de juiste vragen gesteld werden. Je kreeg wel wat van mijn punten en komma’s die altijd op de verkeerde plaats stonden, of er helemaal niet stonden, ooit sal alles reg kom. Je rust, hulp en liefde voor de
immunologie heeft mij enthousiast gemaakt voor de immunologische aspekten binnen de kinderonderzoek, waarvoor mijn hartelijke dank.

Lieve Leontien. Jij was mijn “partner in crime” wat de systematic reviews betreft. We hebben samen wat afgeploeterd met het SDD stuk. Samen op een zondag in jouw tuin in Zeist, dat vond ik heel bijzonder. Zonder jouw hulp met de analyses en het 10x nakijken van de data was het geen stuk geworden, dat nu in ieder geval bij de Lancet is “gesubmit”, we gaan duimen dat het daar wordt aangenomen. Nu ben je coordinating editor van de Cochrane childhood cancer review group, en gaan we samen zorgen dat er nog veel meer systematic reviews in de kinderonderzoek komen. Dank voor al je hulp Leontien.

Dan de andere leden van de promotie commissie.

Beste Prof. Hesseling. Met mijn achtergrond van mijn kindergeneeskunde opleiding in Zuid-Afrika en een mastersproject dat nu het 2e hoofdstuk is van het boekje, was het voor mij niet een volledige promotie als er niet iemand bij was uit Zuid-Afrika, en daar bent u de aangewezen persoon voor. U bent de professor waar ik ook mijn FCP-pediatrics II examen bij heb gedaan en vreselijk streng vond toen. Tijdens alle SIOP congressen heb ik u beter mogen leren kennen, en ben ik zeer vereerd dat u in mijn promotie commissie plaats heeft genomen. Baie baie dankie, en geniet die tulpe in die lente in Holland.


Beste Prof. Heymans, beste Hugo. Je enthousiasme over de uitwisseling van arts-assistenten naar Kaapstad kan ik zeer waarderen, en samen hebben we ook wel eens zitten brainstormen over de opleiding in Zuid-Afrika. Het is voor mij een eer je in de promotie commissie te hebben, en kijk uit naar de discussies op 23 april.

Beste Prof. Offringa, beste Martin. Toen ik in 1997 in het AMC begon was de eerste cursus die ik mocht volgen een EBM(evidence based medicine)course. Jij hebt me geïntroduceerd in deze techniek en ervoor enthousiast gemaakt, en aangemoedigd om een Cochrane review te doen. Dank voor je tips, dank voor alle bemoedigende woorden, en dank voor het plaats nemen in de promotie commissie.

Beste Prof. Speelman. Sinds mijn AMC tijd bezoek ik ieder jaar trouw het infectie symposium en daar heb ik een enorme bewondering voor u ontwikkeld. Een persoon met een brede ervaring in de infectiologie en een goede klinicus. Bij het laatste symposium hield u een lezing
over de behandeling van Clostridium difficile infecties, wat natuurlijk mooi aansluit bij mijn neutropene enterolitis artikel. Dank voor het plaats nemen in de promotie commissie.

Dan alle mensen die mee hebben gewerkt aan de verschillende onderzoeken...

Jan Tamninau: Het neutropene enterocolitis onderzoek was niet mogelijk geweest zonder de input van de Kinder-gastroenterologie (Bert Derkx, Diderik Bosman, Hankje Escher, Michelle Zuckermann, Mark Benninga). Het was jou idée om het prospectief te onderzoeken met rectoscopieën bij deze kinderen. Altijd even enthousiast over het uitwerken van nieuwe ideeën of het zoeken in de literatuur naar een pathofysiologische verklaring, dat heb ik heel erg gewaardeerd. Ik hoop dat we straks verder kunnen met interventie-studies voor de neutropene enterocolitis. Dank voor het altijd klaar staan, ook al was het vrijdagmiddag 5 uur...

Prof. ten Kate: Alle microscopie van de NEC studie is door u verricht. Met het opschrijven van het case-report heeft u me geïntroduceerd in de microscopie van de neutropene enterocolitis. Uw kamer ligt helemaal vol met microscopie glasjes maar u weet er altijd de goede uit te halen, dank voor alle hulp.

Lodewijk Spanjaard: we begonnen met discussies over het opzetten van een klinische trial over het toedienen van antibiotica voor insertie van de centraal veneuze lijn, later werd het een systematic review. Dank voor het meedenken en discussieren over kweken, kwantitatief of kwalitatief en over wat een goed antibiotica beleid is in het oncologische kind. Jou betrokkenheid bij de kliniek heb ik altijd zeer gewaardeerd.

Maarten Biezeveld: Alle immunologische bepalingen van het neutropene enterocolitis onderzoek werden door jou gedaan op het CLB. Ik kon er altijd op vertrouwen de data op tijd terug te hebben. Heel hartelijk dank voor je hulp. Succes met de afronding van je eigen proefschrift en proficiat met je plaats voor de kindergeneesekunde opleiding.

Job van Woensel: Voor het selecteren van een 2e reviewer bij de systematic review over lijnen was jij de aangewezen persoon hiervoor. Jij hebt me geleerd wat minder fel tegen kritiek van reviewers aan te kijken en het vooral rustig en opbouwend te beantwoorden, dank en veel succes met je 2e Cochrane review met Danielle Blom.

Moniek de Witte:Wat was ik blij Moniek dat jij tijdens je co-assistententijd er zoiets als een systematic review over SDD bij wilde doen. Het was een heel werk, maar je deed het vol enthousiasme en uiteindelijk is het een prachtig stuk geworden. Blijf zo enthousiast als je bent, en heel veel success op het NKI en in je verdere carrière.

Bregtje Lemkes: Samen met Emma hebben jullie een aantal maanden alle data verzameld van alle oncologische patientjes en hun MBL-data. Ik kon altijd op je rekenen Bregtje, vooral toen we ongeveer binnen een week alle data moesten verwerken en een presentatie in elkaar moesten draaien. Het is gelukt met jou hulp. Heel veel succes met de co-schappen en heel erg bedankt.

Mireille Vossen: Het onderzoek om kinderen te vaccineren tegen waterpokken was praktisch lastig uitvoerbaar, maar met de klinische en de virologische data samen is het toch een mooi
artikel geworden. We hebben samen met Taco wat zitten worstelen over de resultaten en de discussie, allemaal in December. Ik heb het heel erg gewaardeerd dat je je aan mijn deadline gehouden hebt en gewoon tussen kerst en oud en nieuw alles samen met mij klaar hebt gemaakt. Echt super. Heel veel succes met je eigen proefschrift, dat gaat zeker lukken.

**Jan Weel.** Dank voor al je input in de waterpokken studie. Veel waardering had ik voor de verbondenheid met de kliniek. Het project gaat nog steeds door maar jij zit ondertussen in Leeuwarden, zonder Pauline Wertheim en Hans Zaaijer zou dat niet gekund hebben. Dank voor al jullie hulp.

**Koert Dolman.** Samen hebben we onze 2 studenten begeleid op het MBL project. Nu gaan we verder met Florien op het MBL-substitutie project. Jou heerlijk spontaan enthousiasme is wat een mens af en toe nodig heeft. Ik waardeer je daarin heel erg en hoop nog veel met je samen te werken.

En natuurlijk het secretariat. Carolien, Katenka, Nienke en Annelize. Alle brieven en geregeld om de publicities de deur uit te krijgen, zonder jullie, en vooral zonder mijn zuid-afrikaanse maatje was dat niet gelukt.

En om het hele proefschrift een mooie lay-out te geven was niet gelukt zonder Chris Bor. Geen gemakkelijke opgave want alle documenten liepen vast, nog nooit meegemaakt, maar het is gelukt, heel hartelijk dank. Inge Kos heeft mij geholpen met de kaft van het boekje. Het is prachtig geworden, mijn zuid afrikaanse achtergrond is er goed in terug te vinden, dank.

**En dan F8Noord.**

Sinds 1997 werk ik met veel plezier met alle “onco-zakken” (Huib, Jan, Henk, Jozsef, Cor, Arnauld en Hans). Samen vormen we een goed team, de een meer op de kliniek de ander meer in het onderzoek. Het laatste half jaar heb ik me noodgedwongen teruggetrokken uit de kliniek om het boekje af te schrijven, dat had niet gekund zonder jullie hulp waarvoor heel hartelijk dank. Vooral Lieve Tijtgat, die nu in Zaandam werkt wil ik extra bedanken omdat zij de zorg had over heel wat van mijn “moeilijke” patientjes. Het Soemalie project is gelukt, dank zij jou. Heel veel succes als opleider in de Heel, ik heb er alle vertrouwen in.

Ook Hans wil ik extra noemen. Mijn maatje op F8 Noord. problemen van de afdeling, problemen met patientjes, we kunnen het altijd bij elkaar kwijt maar ook waren wij de enige 2 niet gepromoveerd op de gang en dat schept heel gauw een band. Samen naar deadlines toewerken, samen statistische problemen oplossen en ook in hetzelfde jaar promoveren. Dank voor alle cappucino’s, broodjes op het plein, en vooral dank voor de bemoedigende woorden, vooral in de laatste maanden. Ik weet zeker dat zowel Hanneke als jij een mooie promotie tegemoet gaan, veel sterkte met de laatste loodjes.

En dan de verpleging... Het is alom bekend dat de verpleging van F8noord goed georganiseerd is en goed functioneert. Dat komt door de feedback iedere middag om 4 uur, luisteren naar
elkaar en m...... Velen van jullie zijn meer dan verpleging voor mij, iedereen kan ik hier niet noemen, maar mensen zoals Jane, Hanneke en Edith kan ik hier niet overslaan. Ik hoop dat we nog heel lang samen zullen werken, en buiten het werk nog veel gezellige etentjes zullen regelen.


Astrid en Yvonne moeten zeker genoemd worden, altijd maar weer statussen vinden, of patientnummers zoeken...... dank

Nelia, het laatste jaar zijn we heel intensief met elkaar opgetrokken. Omdat jij de promotie net gehad hebt kon je me veel tips geven en helpen bepaalde valkuilen te voorkomen. Dank voor je steun, de kopjes thee en dank voor je kleine heel speciale kadotje dat mij door de promotie heen zal helpen.

De poli en dagbehandeling. Ook in het laatste half jaar heb ik wel de poli door gedaan ook al was het op een lager pitje. Het is dan belangrijk dat alles goed is voorbereid door de verpleging. Eerst werden mijn poli’s voorbereid door Wil op dinsdag en Suzanne op woensdag, nu zijn Greet en Suzanne mijn trouwe maatjes. Maar ook Sonja, Vera en alle anderen waren bereid in te springen toen de dinsdag poli niet meer door Wil gedaan werd. Dank voor al het geregeld, voor alle kopjes koffie en thee en voor altijd een luisterend oor.

Vrienden:

Mijn Paronymfen Magda en Ingrid

Magda we kennen elkaar al sinds Nijmegen het eerste jaar psychologie aan de K.U Nijmegen. Heel veel hebben we met elkaar opgetrokken van samen studeren tot samen op vakantie gaan. Nu zien we elkaar minder frequent maar het contact blijft hecht en ik ben dan ook heel blij dat jij op 23 April aan mijn zij zal zitten, ik hoop dat jou psychologische blikken een gunstige uitwerking zullen hebben op de promotie commissie. En heel hartelijk dank voor de organisatie rondom het feest. Ik weet dat ik een onmogelijke wens had, maar ik weet ook dat je je uiterste best hebt gedaan er iets moois van te maken.

Ingrid. Wij kennen elkaar vanaf het eerste jaar medicijnen aan de KU Nijmegen. Achter de microscoop kijkend naar histologie plaatjes is de vriendschap begonnen. Ons grootst project tijdens de studie was de wetenschappelijke stage in het buitenland regelen, en wat hebben we een gouden tijd gehad in Iowa. Na de studie ging ik naar Zuid-Afrika, en vond ik het geweldig dat je me daar op kwam zoeken, en hebben we samen een heerlijke vakantie gehad. Nu werk je als gynaecoloog in Rotterdam en ben je nu ook serieus bezig zo’n boekje in elkaar te draaien, knap hoor met 2 kleine kinderen om je heen. Ingrid ik vind het een heerlijk vertrouwd gevoel dat jij mij bij zal staan als paronymf, en dank voor je organisatie rondom het feest.

Cathy. I have known you from the first year I started as a senior house officer in pediatrics at
the Johannesburg hospital. You and your family became my family, and I won’t forget the Sunday afternoon lunch and relaxing at the pool. We did our whole registrar time together and although you graduated before me you kept phoning me at 10 at night to find out how the study was going. Then I went back to the Netherlands and you followed a year later and worked as a neonatologist in Leiden. I really thought you had become a ‘cloggie’ and wouldn’t leave Holland, but you are in Aberdeen now, where you can talk English again. I miss you in Cloggyland and I hope sincerely that you will be there on the 23rd of April.

Paulien. I have known you since I was about 3 years old... For me you are my big sister, the sister I never had. You were always the one who wanted me to enjoy life and not study so hard. Even as a little girl you would tease me and make me swim before doing my home-work. Recently you spent a few years in Holland. I was very pleased you were close to me, but I could see you were not happy here. Now you are back in good old SA. I miss you here, but I’m pleased that you are happier now. I really wish you could be here on the 23rd of April. Thanks Paulien for the help with the cover of the thesis, it is really appreciated.

Moira: We grew up together in Durban. Because we are the same age we thought of each other as sisters. Although our lives separated we always kept in close contact with each other, and during the 7 years I spent in Johannesburg you and your family were my home away from home. The day you told me Hayden would be my Godson was very special for me. I’m sorry you will not be there in April, but I will come and tell you all about it in May. Thanks for always being there.

Margreet. Wat vind ik het erg dat je deze dag niet samen met mij kan meemaken.

Ik had veel bewondering voor je als mens en als collega. De kracht waarmee jij in het leven stond en de laatste jaren je ziekte gedragen hebt was heel bijzonder. De spreuk op je graf past heel goed bij jou “As long as men will breathe or eyes can see, so long lives this and this gives life to thee” (W.Shakespeare). Ik hoop dat je ergens bij me zal zijn op 23 april.

Ellen, eerst kende ik je als secretaresse van Tom, later werden we vriendinnen van elkaar. Dank voor alle gezellige etentjes, het uurtje ontspanning iedere week, zonder jou ging ik niet zwemmen, en dank dat je even mijn prive secretaresse wilde zijn om alle adressen goed georganiseerd te krijgen. Nu kun je ook weer eens bij mij komen eten.... En dat laatste geldt voor alle andere vrienden. De etentjes waren allemaal afgelast want Marian had geen tijd. Ik hoop dat daar verandering in komt. Ciska, Tecla en Jacqueline we moeten nodig wat afspreken, ook al missen we Margreet nog vreselijk. De stelling over vrienden in mijn proefschrift is bewust gekozen omdat ik heel dankbaar ben voor de club vrienden om mij heen, die mij helpen, liefde geven en vertrouwen hebben in alles wat ik doe. Dank....

Lieve pappa en mamma, het proefschrift is aan jullie opgedragen omdat ik heel blij en dankbaar ben dat jullie er allebei bij zijn. Zo vaak zei pappa tegen mij, komt dat proefschrift nou nog eens af, ik wil het wel meemaken. Het is gelukt, en jullie zitten beiden op de eerste rij in de Lutherse kerk. Als enig kind heb ik altijd alle aandacht gehad en werd het mij mogelijk gemaakt in 2 landen.
op te groeien. Door jou beroep pappa (kapitein grote handelsvaart) werd het mij mogelijk gemaakt al op jonge leeftijd veel van de wereld te zien. Dat geeft een verrijking van het leven. Dank voor jullie vertrouwen in mij, en dat jullie mij de gelegenheid hebben gegeven zover te komen...
And last but not least mijn allerliefste Chris. Zoveel jaren kennen we elkaar al maar er was een congres in Australië voor nodig in 2001 om te beseffen dat we zover van elkaar niet zonder elkaar verder wilden. Jou steun, liefde, begrip en gezelligheid is hier niet in woorden uit te drukken. Ik hoop dat we samen een lange toekomst tegemoet gaan en ik ben heel trots en dankbaar dat je deze periode in mijn leven mee hebt mogen beleven.
Verder heb je mijn leven verrijkt met jouw kinderen, Constantijn, Christiaan, Elisah, Jasmijn en Amber. Het is een voorrecht te delen in hun leven, en de tiener perikelen van dichtbij mee te maken... Amber 14 jaar vroeg op een keer “wat doe je toch altijd achter de computer”, ik vertelde haar dat ik een boekje schreef en toonde haar een ander proefschrift. Ze vroeg mij “vind je dat leuk?? “, daar moest ik over nadenken, en antwoordde ik “als het af is is het leuk“ want dan geef je een groot feest, en dan mag jij ook komen. Nou dat was goed, dus Amber geniet ervan.... En wel een heel bijzonder plekje in mijn hart heeft je oudste zoon David, die op 4 jarige leeftijd in het EKZ is overleden aan een infectie tijdens de behandeling voor leukemie. Ik zat toen nog op de middelbare school en voor mij was dit mannetje een trigger om eerst in het EKZ stage te lopen tijdens de studie Geneeskunde, maar ook om de richting van de kinder-oncologie uit te gaan. Nu werk ik als kinder-oncoloog in het EKZ en promoveer ik op infectieuze complicaties tijdens de behandeling voor kanker, zodat kinderen zoals David hier profijt van zullen hebben en minder ernstige infecties zullen krijgen en hopenlijk niet meer aan infecties zullen overlijden. Op de toekomst van al onze patientjes....
En op onze toekomst samen Chris........
Hambani Kahle
“Goodbye and go well all of you”
Stellingen behorende bij het Proefschrift van M.D. van de Wetering

❖ Langdurige opnames van patientjes met kanker in Zuid-Afrika leiden tot infecties met resistent organisme, vooral Gram-negatieve en schimmels. *(dit proefschrift)*

❖ Prophylactische antibiotica voor het inbrengen van een central veneuze lijn is aan te raden bij oncologische kinderen met een infectie risico van meer dan 10%. *(dit proefschrift)*

❖ SDD leidt niet alleen tot vermindering van het aantal Gram-negatieve infecties, maar ook de sterfte ten gevolge van infecties zal door SDD verminderen. *(dit proefschrift)*

❖ Het op juiste wijze uitvoeren van een systematische review geeft antwoord op goed geformuleerde klinische vraagstukken. *(dit proefschrift)*

❖ There are many doctors who are convinced GOBSAT evidence (good old boys sitting around the table) is the best available evidence. *(BMJ 1999)*

❖ Interleukine-8 is een goede voorspeller voor de ernst van het beloop van neutropene enterocolitis. *(dit proefschrift)*

❖ Betere communicatie tussen arts en patiënt zou heel wat leed kunnen voorkomen. *(actueel)*

❖ Voor een goede communicatie geldt dat de zender verantwoordelijk dient te zijn voor een goede ontvangst. *(actueel)*

❖ Er moet een goede balans zijn tussen evidence based medicine en patient centered medicine. *(Prof.Dr.J Bensing, gezondheids psycholoog in arts en auto Feb 2004)*

❖ Life is like a box of chocolates, you never know what you are going to get. *(Forest Gump)*

❖ Many people will walk in and out of your life, but only true friends will leave footprints in your heart. *(Eleonor Roosevelt)*

❖ We can do no great things, only small things with great love *(Danielle Vernooy 1984-2003)*

❖ Kinderen zijn zo bijzonder omdat ze het vermogen hebben van het leven te genieten, daar kunnen we als volwassene veel van leren. *(Wayne Dyer, psycholoog in “bloemen langs de weg”).*