Hematopoietic stem cell transplantation (HSCT) provides effective treatment of hematological malignancies and other disorders. However, the procedure temporarily compromises the immune system resulting in damage to the gastrointestinal (GI) tract, called mucosal barrier injury (MBI), and neutropenia. The GI tract is host to billions of micro-organisms that not only share our body space but are essential for health. These micro-organisms constitute the microbiome and seldom cause us harm. However infection can, and does, occur when the mucosal barrier of the gut is injured. Fortunately antimicrobial agents are employed to prevent and treat infectious complications.

Never the less, as this thesis shows, almost all HSCT recipients develop fever that is the result of inflammation induced by certain drugs employed to prepare for the transplant. Furthermore, measuring the blood concentrations of the amino acid citrulline provides a means of assessing MBI and indicates that MBI, rather than neutropenia, defines the period of risk of fever and bacteraemia following transplant. With the ready availability of blood, this simple and reliable test can help to explore ways of ameliorating MBI to reduce inflammation and fever which, in turn, would lead to a more tailored approach to antibiotic treatment. This can only be a good thing as it will help reduce the risk of antimicrobial resistance.

Knowing the citrulline level could also help decide whether a patient needs to be admitted to hospital or can be treated safely at home.
The role of citrulline
in patients following hematopoietic stem cell transplantation

Alexandra H. E. Herbers
The research presented in this thesis was performed at the Department of Hematology at the Radboud university medical center, Nijmegen, The Netherlands.

Graphic design: J Ontwerp, Nijmegen, The Netherlands

The research performed in chapter 8 and 9 was financially supported by unrestricted educational grants of: Fonds NutsOhra (chapter 8) and Amgen (chapter 9).
Financial support for printing of this thesis was kindly provided by: Radboudmc Nijmegen; Amgen; Bayer HealthCare; Novartis Oncology; Pfizer, Roche Nederland; Mundipharma.

© 2015 Alexandra H. E. Herbers.
All rights are reserved. No part of this thesis may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the written permission of the author. The copyright of the publications remains with the publishers.
The role of citrulline in patients following hematopoietic stem cell transplantation

Proefschrift

ter verkrijging van de graad van doctor

aan de Radboud Universiteit Nijmegen

op gezag van de rector magnificus prof. dr. Th. L. M. Engelen,

volgens besluit van het college van decanen

in het openbaar te verdedigen

op vrijdag 21 augustus 2015

om 14.30 uur precies

door

Alexandra Helena Elisabeth Herbers

geboren op 13 december 1974

te Harderwijk
PROMOTOR
Prof. dr. N. M. A. Blijlevens

COPROMOTOREN
Dr. W. J. F. M. van der Velden
Dr. J. P. Donnelly

MANUSCRIPTCOMMISSIE
Prof. dr. J. G. van der Hoeven
Prof. dr. J. P. H. Drenth
Prof. dr. E. S. J. M. de Bont (UMCG)
Voor Hélène Even
(1974-2007)
Contents

Chapter 1  General introduction and outline of the thesis

Background of managing febrile neutropenia in hematology patients

Chapter 2  Managing febrile episodes in neutropenic patients with hematological malignancies
Managing Infections in Patients with Hematological Malignancies 2009

Introduction of citrulline as biomarker for intestinal mucosal barrier injury

Chapter 3  Citrulline as a biomarker for intestinal mucosal barrier injury (MBI) in hematopoietic stem cell transplant (HSCT) recipients
Submitted

Chapter 4  Citrulline-based assessment score: first choice for measuring and monitoring intestinal MBI in HSCT recipients
Annals of Oncology 2010

Chapter 5  Citrulline and albumin as biomarkers for intestinal MBI in recipients of HSCT
Bone Marrow Transplant 2013

Febrile neutropenia or febrile mucositis?

Chapter 6  Bacteraemia coincides with low citrulline concentrations after high-dose melphalan in autologous HSCT recipients
Bone Marrow Transplant 2008

Chapter 7  Intestinal MBI determines the inflammatory response and early complications in patients receiving conditioning for a HSCT
PLoS One 2010

Chapter 8  Mucositis not neutropenia determines bacteraemia among HSCT recipients
Transplant Infectious Diseases 2014
Consequences of using citrulline in clinical practice

Chapter 9  Impact of palifermin on intestinal MBI of HSCT recipients after BEAM
           Bone Marrow Transplant 2014

Chapter 10 Clever planning of HSCT avoids febrile complications at the weekend
           Submitted

Chapter 11 MBI, fever and infections in neutropenic patients with cancer,
            introducing the paradigm febrile mucositis
           British Journal of Haematology 2014

Chapter 12 Summary and future perspectives

Chapter 13 Dutch summary/ Nederlandse samenvatting voor niet ingewijden

Appendices

Appendix A  List of abbreviations/ Afkortingen
Appendix B  Acknowledgements/ Dankwoord
Appendix C  About the author/ Biografie
            List of publications/ Publicatielijst
CHAPTER 1

GENERAL INTRODUCTION
AND OUTLINE OF THE
THESIS
Chapter 1

GENERAL INTRODUCTION

For many patients intensive treatment with high-dose chemotherapy alone or combined with radiotherapy followed by a hematopoietic stem cell transplantation (HSCT) is the only way to cure hematological malignancies. The supportive care afforded by the transfusion of blood, platelets and stem cell products, treatment with antimicrobial agents and recombinant growth factors, has permitted the use of more intensive regimens, but fever and infection remain the most prominent complications of cytotoxic therapy. Approximately four of every five patients experience fever. Microbiologically documented infections, usually bacteraemia accounts for about one third to half of fevers, clinically documented infections for a further one in five with no infectious aetiology being identified for the remainder. Bacteraemia can result in fulminant sepsis and death and is reported as the primary cause of death of 7% of autologous stem cell transplant recipients and 13-17% of allogeneic stem cell transplant recipients. Damage to the host defences induced by the intensive treatment to prepare for a HSCT is the primary cause of fever and breaches to the innate immunity allow infection and translocation of bacteria into the blood stream leading to infection. The patient’s vulnerable condition warrants prompt administration of empirical antibiotic treatment directed primarily against the gram-negative bacilli that inhabit the gastro-intestinal (GI) tract e.g. *Escherichia coli* and other enteric bacteria as well as *Pseudomonas aeruginosa* at the first occurrence of fever, even if there is no obvious cause for the fever. Nowadays protocols advise treatment with broad-spectrum antibiotics of the third or fourth generation cephalosporins e.g. ceftazidime, carbapenems e.g. meropenem or ureidopenicillins combined with a beta-lactamase inhibitor e.g. piperacillin-tazobactam. Fever persisting for more than 3-4 days is often a reason for adding further antimicrobial agents even when no infectious focus is found. (However our practice is that this should only be done when there is an objectively verifiable reason for doing so). This means that fever and infections bring about the use of increased supportive care, including antimicrobials, prolonged hospitalization and increased costs. Antibiotic treatment leads to selective resistance of the patient’s microflora also called its microbiome. The emergence of antimicrobial resistance with at the same time a decline in the development of new antimicrobial agents can increase the global burden of infectious disease that can threaten the care for hematology patients in the future.

MAN AND HIS MICROBES: A DELICATE BALANCE

The surfaces of the human body internally and externally are covered in billions of individual micro-organisms. There being 10 times the number of normal bacterial cells that live on the body, i.e. approximately $10^{14}$, than cells that make up the human body (Figure 1).
Under normal circumstances these resident microbiota do little harm and, in fact, help protect the human body from becoming infected with harmful microbes. Together with the anatomic barrier (such as skin or GI tract) they prevent the entrance of pathogens into the body. Should bacteria invade, they will be met by neutrophils that gather at the entry side in order to clear the invasion. If these phagocytes fail, other blood cells of the innate and adaptive immune system are ready to come into action.

When cytotoxic therapy damages the host defences, the skin and alimentary tract mucosa become a reservoir of potential opportunistic pathogens. Furthermore, the intensive treatment will also disrupt the balance between the host and microbial residents. Not surprisingly, infections of HSCT recipients are typically due to those opportunistic pathogens that normally inhabit the surfaces of the human body, rather than professional pathogens such as *Streptococcus pneumoniae*. Indeed, the common causes of infection during neutropenia following HSCT are due to bacteria including Gram-negative bacilli e.g. *Escherichia coli*, and less frequently *Pseudomonas aeruginosa* as well as gram-positive cocci especially the so-called coagulase-negative staphylococci e.g. *Staphylococcus epidermidis*, and oral viridans streptococci e.g. *Streptococcus mitis*. Fungal infections occur at a rate of < 10% and involve mainly yeasts, e.g. *Candida albicans* and moulds e.g. *Aspergillus fumigatus*. Viral infections, especially Herpes simplex virus are also common. Never the less, mortality is mainly associated with severe bacterial sepsis, pneumonia and invasive fungal infections.
Chapter 1

CYTOTOXIC TREATMENT INDUCED DAMAGE TO HOST DEFENCE MECHANISMS IN HSCT RECIPIENTS

1. NEUTROPENIA
Historically the focus has been on neutropenia as main risk factor for the occurrence of fever and infections in patients with cancer. The myeloablative nature of chemotherapy results in bone marrow suppression shortly after cytotoxic treatment is given leading to a precipitous decrease in circulating blood cells. Neutrophils are particularly affected because of their short life span, resulting in neutropenia which is typically defined by an absolute neutrophil count of ≤ 0.5 x 10⁹ granulocytes/L. Hence, measures to prevent infectious complications are determined on the onset, depth and course of neutropenia. Fever is often the first and only sign of infection in HSCT recipients and when this develops during neutropenia, it is called “febrile neutropenia” and represents a signal to start empirical antibiotic therapy promptly in order to prevent the progression of the putative infection to sepsis and even death. Moreover, nursing personnel undertake extra controls and monitoring based on the duration of neutropenia and, in many centres, antibiotic therapy is often continued until neutropenia resolves.

2. DAMAGED MUCOCUTANEOUS BARRIERS
The high rate of division and turnover of cells of the skin and alimentary tract renders them particularly vulnerable to damage induced by the intensive chemotherapy given prior to a HSCT. Since the gut is the largest reservoir of the commensal flora, damage there will play an important role in the occurrence of infection and fever in HSCT recipients. Surprisingly cytotoxic therapy-induced damage to the GI tract has attracted little attention until recently.

2.1 Mucositis or mucosal barrier injury
Cytotoxic therapy-induced damage to the mucosal barrier of the alimentary tract is called mucosal barrier injury (MBI) and mucositis refers to the attributable clinical manifestations. MBI affects the entire alimentary tract and is often separated into oral MBI and intestinal MBI based on anatomical and functional differences. MBI is seen as one of the most debilitating side-effects of the myeloablative treatment employed for a HSCT though it eventually resolves spontaneously. Most knowledge regarding MBI has been garnered from oral MBI since the manifestations can be easily observed.

2.2 Pathogenesis of MBI
The development of MBI depends on the type of cytotoxic treatment, the agents employed, and the dose and duration of the regimen. Virtually every patient who receives a myeloablative regimen for a HSCT develops MBI to some extent and the incidence for reduced intensity regimens for MBI is approximately 40-50%.
According to the model introduced by Sonis, five overlapping phases are important in the pathobiology of MBI.\textsuperscript{28} (1) Initiation phase, in this phase the intensive treatment given prior to a HSCT induces both DNA and non DNA damage and injury in cells of mucosa as well as submucosa occurs. Generation of reactive oxygen species leads to the activation of nuclear factor-kappa B (NFkB).\textsuperscript{29} In the (2) upregulation and message generation phase, DNA- and non DNA-damage triggers a cascade of biological events. Several transduction factors are upregulated, including NFkB leading to the release of cytokines and chemokines (IL-1β, IL-6, IL-8, TNFα, IL-23, interferon-gamma (IFNγ)) by tissue macrophages, dendritic cells, and release of danger-associated molecular patterns (DAMPs). Furthermore the secretion of matrix metalloproteinases is stimulated. The result is mucosal injury and death.\textsuperscript{30,31} In the (3) amplification and signalling phase, tissue injury is accelerated and amplified through feedback loops.\textsuperscript{32} This leads to the (4) ulcerative phase where breeches in the mucosal epithelium may provide a portal of entry for bacteria, viruses and fungi that reside on the surface. Microbes and pathogen-associated molecular patterns (PAMPS) activate immune cells, stimulate the production of pro-inflammatory cytokines, leading to further inflammation and apoptosis.\textsuperscript{33-35} Finally, (5) healing occurs, under the influence of several extracellular matrix signals that affect the rate of epithelial cell migration, proliferation and differentiation. Recent research also suggests that the deregulation of the microbial homeostasis by cytotoxic treatment could influence each one of the phases of this model.\textsuperscript{36} Although developed for oral MBI this model is also likely to be applicable to intestinal MBI though the events that take place in the intestine are almost certainly more complicated than those occurring in the oral cavity not least because of the plethora of microbial species that inhabit the human gut.\textsuperscript{28,37-40} These bacteria can influence the course of MBI as can gut function in terms of nutrient digestion and absorption of food after ingestion.\textsuperscript{41,42} The gut also has an enormous surface area with the small intestine alone having an estimated surface area 20 times that of the skin.\textsuperscript{43}

2.3 Structure of the small intestine
The small intestine comprises three functionally distinct segments: the duodenum, jejunum and ileum. The structure of the mucous membrane lining the intestine is similar in all three parts. The small intestine wall is composed of four layers: the mucosa, submucosa, muscularis externa and serosa. The inner surface of the small intestine is covered with circular folds or valves of Kerckring which are composed of the mucosa and submucosa (Figure 2). The valves of Kerckring are positioned perpendicular to the long axis of the intestine and project into the intestinal lumen. These structures increase the absorptive surface area 2-3 fold and are most prominent in the distal duodenum and jejunum.
The mucosa of the small intestine consists of three layers: epithelium (innermost layer, facing the intestinal lumen), lamina propria mucosae (connective tissue) and muscularis mucosae (smooth muscle). The epithelium is made of a monolayer of numerous invaginations (crypts of Lieberkühn) and finger-like protrusions (villi). Their role is to further increase the absorptive surface of the intestine, by a factor of approximately 10 (yielding 30-fold increase in total) (Figure 2). Almost 90% of the cells comprising the epithelium are enterocytes which are responsible for the degradation and absorption of food after ingestion. Their luminal surface area is covered with microvilli. The presence of microvilli increases the mucosal surface area of the intestine still further by a factor of approximately 20-30 fold resulting in 400-900 fold increase in total (Figure 3).

**Figure 2. Valves of Kerckring**

![Valves of Kerckring](image)

1. Epithelium, 2. lamina propria mucosae, 3. muscularis mucosae

*Mucosa of the small intestine*

The mucosa of the small intestine forms one of hierarchical tissues. There are three types of cells: stem cells of high proliferative activity, incompletely differentiated daughter cells and mature fully differentiated cells. Complete renewal in the small intestine requires 4 to 6 days.
The stem cells in the lower half of the crypts give rise to daughter cells, thereby producing all the cells of the epithelium. Newly produced cells migrate and differentiate into four principal cell types. Most cells differentiate into functional enterocytes, goblet cells (± 4%, production of mucus) and enteroendocrine cells (± 0.5%, production of hormones) and migrate up along the villi to the surface of the intestinal lumen. The migration of these new cells continues until they reach the tips of the villi, where they undergo apoptosis and are shed into the lumen. The fourth cell type, Paneth cells (± 7.5%, production of defensins that are important for immunity) migrate downward and reside at the bottom of the crypt (Figure 3).

Due to their high turnover rate, the mucosal cells of the intestine are particularly vulnerable to cytotoxic therapy. The proliferative stem cells are very sensitive to DNA damage and are often killed by the intensive treatment used for HSCT. The daughter cells are less vulnerable to cytotoxic therapy-induced damage and are able to repair this damage, retain stem cell properties and can repopulate the crypt.
2.4 MBI, inflammation and infection

Cytotoxic therapy-induced damage to the intestinal mucosa induces mucosal inflammation, as explained in the Sonis’s model.\textsuperscript{28,39} The onset of mucosal inflammation is determined by the life-span of mature epithelial cells. Once the cytotoxicity ceases, repopulation of stem cells and daughter cells ensues resulting in complete recovery and cessation of inflammation.\textsuperscript{49} The duration and intensity of mucosal inflammation is determined by the direct toxic effect of the intensive treatment on the mucosa together with the inflammation resulting from the imbalance between the microbiome and the immunocompromised host.\textsuperscript{24} MBI can induce infection due to the disruption of the body’s natural anatomic barrier allowing opportunistic pathogens to translocate to the bloodstream, especially when also the immune system is severely compromised.\textsuperscript{49,50}

To examine the role of mucosal inflammation and translocation of pathogens due to MBI in the occurrence of fever and infections, it is first necessary to gain an impression of the severity of the mucosal damage that occurs after the intensive treatment is given.

2.4.1 Scoring methods for MBI

Several assessment methods are applied.

\textit{A. Based on signs and symptoms:}

Oral MBI is visible in the oropharynx as red, inflamed, easily bleeding tissue with or without the presence of ulcers. Symptoms include pain, oedema, excessive mucus production, reduced saliva and haemorrhage all which reduces the patient’s ability to eat and drink.\textsuperscript{51,52} Assessment scorings systems, including the World Health Organization and the National Cancer Institute-Common Terminology Criteria for Adverse Event (NCI-CTCAE), combine objective signs (erythema and ulcer formation) with subjective and functional outcome (pain and the ability to eat) to gauge the severity of oral MBI.\textsuperscript{53,54}

Intestinal MBI presents a challenge as it cannot be seen directly or readily detected. The intestinal symptoms that can be associated with gastrointestinal MBI include nausea, vomiting, abdominal pain, cramping and watery diarrhoea occasionally accompanied by macroscopic blood loss.\textsuperscript{24} These symptoms are clearly non-specific as they can also be caused or influenced by the conditioning chemotherapy, infection, as well as by medications such as opioids, making assessment scores based on signs and symptoms much less reliable. Intestinal MBI can only be seen by endoscopy and biopsy but these are usually precluded because of the high likelihood for bleeding complications due to the profound thrombocytopenia that develops contemporaneously with bone marrow aplasia.\textsuperscript{54}

\textit{B. Absorption and permeability tests}

The pathological alterations in the intestine due to cytotoxic damage start with crypt loss. This is followed by villous atrophy and blunting, enterocyte damage, infiltration of inflammatory
cells and accumulation of goblet cells at tops of the villi. The end result is gut dysfunction so functional tests have been employed to determine the severity and course of the mucosal damage. Since the principal features of intestinal MBI are loss of epithelial surface and change in permeability several permeability and absorption tests were assessed. The permeability of $^{51}$Cr-labeled ethylenediaminetetracetic acid ($^{51}$Cr-EDTA) will increase shortly after the start of cytotoxic treatment prior to a HSCT. However, $^{51}$Cr-EDTA is radioactive and therefore not suitable for routine use. Sugar permeability and absorption tests have been shown to be able to determine the onset of disruption and dysfunction of the mucosal barrier of the small intestine.

The peak of gut permeability has been shown to occur 10-14 days after starting intensive chemotherapy. However, no real difference could be found in the overall pattern of perturbed gut integrity and the absorptive capacity of patients given various myeloablative regimens. Furthermore, both $^{51}$Cr-EDTA and sugar permeability tests are influenced by extraneous factors such as bowel transit time, gastric emptying and renal function. They are also cumbersome and wholly dependent on compliance, which means that patients are reluctant or not fit enough to undergo these tests when they are feeling particularly unwell or are ill.

A test to measure the intestinal MBI that could be performed in blood would therefore be much better. This would prove less of a burden to the patient especially if the blood has already been taken for other purposes, often via an intravascular catheter.

**C. Citrulline**

In 2000, Crenn et al. reported that the amino acid citrulline was a reliable biochemical marker of the small bowel enterocyte mass of patients. The name citrulline comes from *Citrullus*, the Latin word for water melon. In 1914 Koga and Odake detected it in water melon juice. In 1930 Wada defined the chemical formula of the amino acid and named it citrulline. Diet is a poor source of citrulline and most citrulline is produced by endogenous synthesis predominately in the gut enterocytes of the small intestine, most likely from the middle and upper parts of the villi.

The substrates for citrulline in the small intestine are dietary glutamine, glutamate and proline, and also glutamine found in the serum. In fact, 80% of the circulating citrulline is derived from the conversion of glutamine extracted from the blood.

Five mitochondrial enzymes are involved in the synthesis of citrulline from glutamine in the enterocyte (Figure 4). Glutaminase (GLNase) converts glutamine to glutamate and ammonia. Glutamate is then converted to pyrroline-5-carboxylate by pyrroline-5-carboxylate synthase (P5Cs). P5Cs is converted to ornithine by ornithine aminotransferase (OAT). Glutamine derived ammonia plus bicarbonate (HCO3) are converted to carbamoyl phosphate by carbamoyl phosphate synthetase 1 (CPS1). Carbamoyl phosphate and ornithine are finally converted to citrulline by ornithine carbamoyltransferase (OCT).

Proline is another precursor for pyrroline-5-carboxylate. Via proline oxidase (PO) proline is converted into pyrroline-5-carboxylate. The subsequent metabolic steps are identical as for the citrulline synthesis from glutamine and involve OAT, CPS1 and OCT.
Although small intestinal enterocytes contain a complete urea cycle, there is a very low level of argininosuccinate synthetase (ASS) in the enterocyte so, as a result, citrulline is released in the bloodstream.\(^{66,74}\)

**Figure 4.** Citrulline synthesis in small intestinal enterocyte

The availability of substrate and the activity of the intestinal citrulline synthesizing enzymes can influence the citrulline concentration in plasma but it has been shown in preclinical experiments and confirmed by clinical data that the number of functional enterocytes is the major determinant for the release of citrulline into the circulation.\(^{63,74-78}\)

After citrulline is released into the bloodstream it passes through the liver nearly unchanged and is not influenced by systemic inflammation. It is removed from the blood by the kidney, where it is converted to arginine. Hence the concentration of citrulline depends only on the production and release of citrulline by the enterocytes mass and on renal excretion.\(^{79}\) Individuals with normal intestinal mucosal function and normal renal function given a Western diet have citrulline levels between 30 and 50 \(\mu\text{mol/L}\) with a median of 40 \(\mu\text{mol/L}\), as determined by ion-exchange chromatography.\(^{66}\) The citrulline concentration in blood will decrease when there is
injury to the intestine, but has no intrinsic diagnostic value. Regular assessment of citrulline allows monitoring of small intestinal function the only limitation being marked renal failure (creatinine clearance < 30 ml/L).\textsuperscript{66}

In patients with villous atrophy disease, the plasma level of citrulline has been shown to be discriminative for the degree of villous atrophy: < 10 μmol/L corresponding with total villous atrophy, levels ranging between 10 and 20 μmol/L corresponding with subtotal villous atrophy and > 20 μmol/L indicating partial atrophy.\textsuperscript{80} Furthermore citrulline can be used for monitoring treatment response. Citrulline concentrations have also proved a reliable marker of the remaining length of the small bowel of patients with short bowel syndrome and are consistent with small bowel absorption capacity.\textsuperscript{63,81,82} Plasma concentrations of citrulline remain low for up to a year after treatment underlining the strict dependence of circulating citrulline on epithelial mass. Furthermore citrulline concentrations are also proposed as marker for patients with small bowel transplants being indicative of the functional epithelial mass or the dependence on total parental nutrition.\textsuperscript{83,84}

In our own hospital citrulline has also been evaluated in HSCT recipients and proved more sensitive and more specific for measuring small bowel enterocyte loss than did sugar permeability tests.\textsuperscript{25,85}

In this thesis the questions of whether citrulline is really able to fulfil its role as valuable and reliable marker for intestinal MBI in HSCT recipients will be explored. Furthermore the relation between intestinal MBI, fever and infection will be examined. Finally, an attempt will be made to see whether knowledge about MBI can lead to changes and improvements in the care of the HSCT recipient.

**OUTLINE OF THE THESIS**

This thesis consists of four parts:

**PART I: BACKGROUND OF MANAGING FEBRILE NEUTROPENIA IN HEMATOLOGY PATIENTS**

In this first part we illustrate the management of febrile episodes in neutropenic hematological patients and show that there is very little interest in the role of MBI in common practice (chapter 2).

**PART II: INTRODUCTION OF CITRULLINE AS BIOMARKER FOR MUCOSAL BARRIER INJURY**

Citrulline is introduced as a marker of MBI that results from the cytotoxic treatment to prepare for hematopoietic stem cell transplants.

In a clinical review we describe various means of measuring MBI and show that citrulline is an
objective, sensitive, precise, validated, reproducible and reliable marker of intestinal MBI in HSCT recipients. We review the history, biochemistry and measurement of citrulline and describe its use and potential utility for managing the intestinal complications arising from treating HSCT recipients (chapter 3).

We examine two different citrulline-based assessment scores for intestinal MBI in a cohort of 94 HSCT recipients (chapter 4).

Next we test whether citrulline is really a more valuable marker of intestinal MBI than serum albumin that clinicians often rely on as indicator (chapter 5).

PART III: FEBRILE NEUTROPENIA OR FEBRILE MUCOSITIS?

In this third part we investigate the relationship of MBI, neutropenia and the incidence of post-transplant complications, especially fever and bacteraemia. Our hypothesis is that MBI, rather than neutropenia, determines the onset and extent of inflammation and influences the incidence of post-transplant complications.

We start by examining the occurrence of bacteraemia in relation to neutropenia and MBI in one regimen (chapter 6).

This is followed by a retrospective study in a cohort of 163 HSCT recipients given five different conditioning regimens, in which the kinetics of citrulline, the relationship of intestinal MBI, and the occurrence of fever and bacteraemia are studied in greater detail (chapter 7).

We further unravel the relationship between intestinal MBI, neutropenia and the occurrence of inflammation, fever and bacteraemia in a study in which a non-myeloablative regimen is compared to myeloablative regimen and blood cultures are taken from admission onwards as well as at the onset of fever (chapter 8).

PART IV: CONSEQUENCES OF USING CITRULLINE IN CLINICAL PRACTICE

In this last part we examine how we might use the knowledge gained from studying MBI and the kinetics of citrulline to improve the management of febrile episodes of patients who received intensive cytotoxic treatment to treat cancer.

Since permeability tests suggest a protective effect of the keratinocyte growth factor palifermin, on the intestinal MBI of HSCT recipients who had received BEAM conditioning, we investigate whether the drug is able to reduce intestinal MBI by monitoring citrulline and evaluate its effect, if any, on the inflammatory response and occurrence of bacteraemia (chapter 9).

Furthermore we examine whether better planning of the start of the intensive treatment before HSCT can prevent recipients from developing inflammatory and infectious complications during the weekend, as this is often accompanied with less than optimal care (chapter 10).

To conclude, we gather together all recent information concerning MBI and show how the inflammatory response and fever occurring in HSCT recipients can be the direct consequence of MBI alone, with neutropenia playing a minor role, if at all. We postulate a new paradigm in the management of febrile episodes in neutropenic cancer patients, namely “febrile mucositis” (chapter 11).
These insights should provide a new impetus for developing drugs that ameliorate MBI, but also should lead to a more targeted use of antimicrobials for the treatment of the febrile HSCT recipient.

REFERENCES


PART I

BACKGROUND OF MANAGING FEBRILE NEUTROPENIA IN HEMATOLOGY PATIENTS
CHAPTER 2

MANAGING FEBRILE EPISODES IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

AHE Herbers and BE de Pauw.
Published as: Acute Myelogenous Leukemia and Febrile Neutropenia.
ABSTRACT

Aggressive chemotherapy has a deleterious effect on all components of the defense system of the human body. The resulting neutropenia as well as mucosal barrier injury allow pathogenic micro-organisms easy access to the body. The symptoms of an incipient infection are usually subtle and limited to unexplained fever due to the absence of granulocytes. This is the reason why prompt administration of antimicrobial agents while waiting for the results of the blood cultures, the so-called empirical approach, became an undisputed standard of care. Gram-negative pathogens remain the principal concern because their virulence accounts for serious morbidity and a high early mortality rate. Three basic intravenous antibiotic regimens have evolved: initial therapy with a single antipseudomonal β-lactam, the so-called monotherapy; a combination of two drugs: a β-lactam with an aminoglycoside, a second β-lactam or a quinolone; and, thirdly, a glycopeptide in addition to β-lactam monotherapy or combination. As there is no single consistently superior empirical regimen, one should consider the local antibiotic susceptibility of bacterial isolates in the selection of the initial antibiotic regimen. Not all febrile neutropenic patients carry the same risk as those with fever only generally respond rapidly, whereas those with a clinically or microbiologically documented infection show a much slower reaction and less favorable response rate.

Once an empirical antibiotic therapy has been started, the patient must be monitored continuously for nonresponse, emergence of secondary infections, adverse effects, and the development of drug-resistant organisms. The average duration of fever in serious infections in eventually successfully treated neutropenic patients is 4-5 days. Adaptations of an antibiotic regimen in a patient who is clearly not responding is relatively straightforward when a micro-organism has been isolated; the results of the cultures, supplemented by susceptibility testing, will assist in selecting the proper antibiotics. The management of febrile patients with pulmonary infiltrates is complex. Bronchoscopy and a high resolution computer-assisted tomographic scan represent the cornerstones of all diagnostic procedures, supplemented by serological tests for relevant viral pathogens and for aspergillosis. Fungi have been found to be responsible for two thirds of all superinfections that may surface during broad-spectrum antibiotic treatment of neutropenic patients. Antibiotic treatment is usually continued for a minimum of 7 days or until culture results indicate that the causative organism has been eradicated and the patient is free of major signs and symptoms. If a persistently neutropenic patient has no complaints and displays no evidence of infection, early watchful cessation of antibiotic therapy or a change to the oral regimen should be considered.
INTRODUCTION

Only 50 years ago, dealing with a patient with a disseminated malignant disease was relatively simple. There were no curative options and information on the inevitable dismal prognosis was often not shared with the patient or his family. The mid sixties of the 20th century witnessed the first successes of chemotherapeutic agents. This encouraged investigators to explore this route further thereby escalating the dosage of the cytostatic drugs in the expectation of better results. It became rapidly clear that the destructive effects of cytotoxic compounds were not limited to malignant cells. Infection has emerged as a prominent complication of chemotherapy, which was particularly worrisome in the sixties, a decade without powerful broad-spectrum antimicrobial agents. Since a possible cure of the cancer was seen as the primary goal, complications of rigid cytotoxic regimens were taken for granted and when they occurred, treatment was more or less improvised. This situation remained actual until Bodey pointed out that patients in remission of their underlying disease could die suddenly of an overwhelming infection during cytotoxic therapy-induced neutropenia. Neutropenia was and remains defined as an absolute neutrophil count of ≤ 0.5 x 10⁹/l (500/ mm³) or a count ≤ 1.0 x 10⁹/l (1000/mm³) expected to fall below 0.5 x 10⁹/l (500/mm³). He even showed a positive correlation between the severity and duration of neutropenia and the risk of acquiring a life-threatening bacterial infection. This risk appeared even more outspoken in individuals who were treated for an acute leukaemia or lymphoma as these disorders interfere directly with vital components of the immune system. Next to gram-negative bacilli, notably Staphylococcus aureus earned a bad reputation. A few years later, Schimpff and coworkers demonstrated convincingly that early administration of antimicrobial agents covering the above mentioned pathogens without waiting for the results of the blood cultures saved lives. His so-called empirical approach became an undisputed standard of care. However, better options to manage infections stimulated the haematologists to intensify their antileukemic regimens further in an attempt to improve the remission rates in refractory cases. Moreover, in the meantime allogeneic bone marrow transplantations have become a fully accepted treatment modality. These intensifications, in turn, inspired a more thorough clinical research of potentially more effective antimicrobial regimens, which was facilitated by the booming development of new antimicrobial agents such as the broad-spectrum penicillins, third and fourth generation cephalosporins, fluoroquinolones, and carbapenems in conjunction with a keen eagerness of the respective pharmaceutical companies to put their compounds to the test in large clinical trials that were usually conducted by cooperative groups. A spiral of several subsequent rounds of improved antibiotic cover and further escalation of chemotherapeutic regimens has eventually downgraded neutropenia to only one of many problems in today’s clinical practice. Modern anti-leukemic therapy is almost inherently associated with ulceration of the mucosa (called mucosal barrier injury (MBI)) allowing micro-organisms originating from the damaged gastrointestinal (GI) tract easy access to the body. These pathogens may be part of the original indigenous flora but are,
more commonly, acquired during hospitalization.\textsuperscript{8} It was, therefore, logic that attempts to
prevent invasion of the body by indigenous flora by prophylactic administration of anti-infective
agents became quite popular. Since such prophylactic agents were mainly targeted against
the gram-negative enterobacteriaceae a shift from gram-negative to gram-positive micro-
organisms, including coagulase-negative staphylococci, viridans streptococci, and enterococci,
as the primary cause of fever in neutropenic patients was seen.\textsuperscript{9-15} In the meantime, therapeutic
regimens in the treatment of hematological malignancies have become so complex that usage
of surgically implanted venous access devices appears inevitable in spite of the apparent risk of
infections and/or thrombosis.\textsuperscript{16,17} An epidemiological survey among hospitalized patients treat-
ed for a hematological malignancy between 1995 and 2001 in the United States showed that
approximately 70 percent (64\% in 1995 and 76\% in 2001) of all microbiologically confirmed
febrile episodes were due to gram-positive bacteria and 18 percent (22\% in 1995 and 14\% in
2001) to gram-negative bacilli.\textsuperscript{18} This change in causative pathogens was enhanced by an
increased use of central venous catheters and other medical devices. Finally, introduction of
immuno-manipulative monoclonal antibodies into the therapeutic arena has extended the
treatment-related immunodeficiency to the T-cell functions and innate immunity, which brought
viral and fungal infections, including \textit{Pneumocystis jirovecii}, into play, particularly when an
impaired cellular community coincided with a prolonged, severe neutropenia.\textsuperscript{19,20} The
modern chemotherapeutic regimens designed to treat acute lymphoblastic leukaemia incor-
porate high doses of corticosteroids. As a result, patients treated with such regimens are at
increased risk of infections typically related to an impaired cellular immunity. Nowadays, in-
fections are still accountable for substantial morbidity and mortality among patients who undergo
myeloablative therapy for a hematological malignancy. In spite of all changes in the spectrum
of infectious agents, gram-negative pathogens remain a principal consideration because of
their virulence that accounts for serious morbidity and a high early mortality rate.\textsuperscript{21,22}

\textbf{MANAGEMENT OF NEW FEVER AND INFECTIONS}

\textbf{PRINCIPLES}

Administration of potentially curative chemotherapy is the starting point; cytotoxic drugs are
given without too much consideration since internationally accepted antitumor protocols
dictate the dosages. Once the chemical compounds are in the body, the haematologist can
relax and wait for the outcome a few weeks later. However, where the scientist in the haema-
tologist has completed his first task, the general clinician in him or her has to step forward
when the natural defence system gradually disintegrates. Indeed, during this episode close
surveillance of the patients with attention for the emergence of infectious complications is
mandatory. It is work on an individual basis, where fixed protocols hardly help.\textsuperscript{23,24} It is here
that the science and art of medicine meet; listening to the patient’s complaints and meticulous physical examinations constitute the crucial factors for timely therapeutic interventions and eventual success. This applies to both patients who are treated with intensive chemotherapeutic regimens and recipients of a stem cell transplant. During this episode appropriate coordination of the information coming from different sources is important, since, next to the patient, family members, nurses, microbiologists, pulmonologists, radiologist and pathologists can assist in the timely discovery of an emerging complication. Different centers acquit themselves from this task in different ways but it occurs to us that the physician who is responsible for treatment of the underlying disease should also act as the captain of the ship. This coordinating role obliges him or her to have at least some basic knowledge of the likely problems and, perhaps even more importantly, to have fine communication skills to keep all parties on board as well as on the same course. Since the symptoms of an incipient infection are usually rather subtle due to the absence of granulocytes, teamwork is crucial to ensure that antibiotics are administered within hours of the first signs or symptoms of infection. In most cases fever, defined as a single oral temperature of more than 38.3°C (101°F) or a temperature of more than 38.0°C (100.5°F) for more than 1 hour, will serve as a trigger for action. At the onset of fever, attempts to identify the cause of fever deserve absolute priority (see Table 1), together with immediate institution of appropriate broad-spectrum antibiotic therapy.

Fever in a neutropenic patient is a warning sign that should be taken very seriously because self-limiting infection is virtually nonexistent in neutropenic patients irrespective whether they have been treated for acute leukemia or lymphoma or did receive a stem cell transplantation. In anticipation of the results of the diagnostic procedures, fever denotes infection until proven otherwise. The absence of phagocytic cells in combination with a damaged integument allows micro-organisms residing at a superficial site of infection easy access to the bloodstream. Under these circumstances, a relatively small inoculum that easily can escape detection when limited volumes of blood are sampled for culturing can cause a serious septic syndrome. Therefore, waiting for a blood culture to become positive is a bad idea, although it should be kept in mind that fever can be of non-infectious origin.

A sudden onset of fever accompanied by chills, tachycardia with or without a drop in blood pressure and tachypnea is associated with a higher rate of positive blood cultures. Shock at the onset of fever is an ominous clinical sign but neither clinical manifestations nor the pattern of fever during neutropenia can serve as an indicator of a particular causative agent, not even when the most notorious pathogens such as *Pseudomonas aeruginosa* or *Staphylococcus aureus* are involved. Acute fever following transfusion is often related to the presence of irregular blood group antigens or to cytotoxic antibodies acquired during previous transfusions or a pregnancy.
### Table 1. Diagnostic procedures at the onset of fever

- Short history of the patient with recent complaints
- Physical examination with special attention for:
  1. vital signs
  2. gastrointestinal tract: peridontium, abdomen, perineum
  3. respiratory tract: oropharyngeal area and lungs
  4. skin, including bone marrow aspiration sites, vascular access sites, and tissue around the nails
- Cultures of blood (minimal 15 ml) and any clinical suspicious body site, including urine
- Radiological examination of the chest
- Check: medication list, compliance with prophylaxis, results surveillance cultures, course of the leukocyte count
- Consider determination of CRP, galactomannan antigen, viral serology and tests for Legionella

A substantial minority of patients will show an insidious onset of fever. Although more frequently related to non-infectious causes than acute fever, a slow rise of temperature does not exclude an infectious origin, although gram-negative rods, viridans streptococci and *Staphylococcus aureus* are less prevalent amongst these patients. Of note, a relative bradycardia in patients who not receive anti-arrhythmic medication suggests either a viral or non-infectious origin of the fever. A possible relation between fever and frequently used drugs such as allopurinol, antibiotics, bleomycin, and cytarabine or with the underlying disease process itself should always be kept in mind. A dysfunctional immune system is presumed to be responsible for the high rate of drug allergy in patients with active acute leukaemia; the allergy may abate when complete remission is achieved. This phenomenon is well known in patients with infectious mononucleosis or acquired immunodeficiency syndrome.

Until recently, coagulase-negative staphylococcal bacteraemia was thought to be entirely related to the use of central venous catheters but recent work points at mucosal sites as an even more important portal of entry. The clinical spectrum of catheter-related infections ranges from asymptomatic bacteraemia as a manifestation of intraluminal colonization or a process confined to the site of insertion to marked inflammation of the tunnel tract and septicemia with metastatic emboli in the skin and other organs. Suspicion of a tunnel or exit line infection should arise when the catheter tract becomes painful, red or swollen or when signs of inflammation are visible at the exit site. Malfunction of the catheter, illustrated by problems to draw blood through the line, is a common first warning of a possible lumen infection.
SELECTION OF ANTIMICROBIAL AGENTS FOR THE EMPIRIC PHASE

BASIC REGIMENS

In the selection of the initial antibiotic regimen, one should contemplate the type, frequency of occurrence, and antibiotic susceptibility of bacterial isolates recovered from other patients at the same hospital. In addition, the use of certain antibiotics may be limited by special circumstances, such as drug allergy, liver function disturbances or renal insufficiency. Despite numerous clinical studies since the 1970s, no single empirical antibiotic regimen has classified as superior for the initial treatment of patients who become febrile during a neutropenic episode after therapy with cytoreductive drugs for a malignant hematological disease (Table 2). However, there is world-wide consensus that any initial antibiotic regimen should include drugs with reliable activity against Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, other enterobacteriaceae, and Staphylococcus aureus.

Three basic intravenous antibiotic regimens have evolved: initial therapy with a single drug, so-called monotherapy; a combination of two drugs, a β-lactam with an aminoglycoside, a second β-lactam or a quinolone but without a glycopeptide; and, thirdly, a glycopeptide as addition to a single β-lactam or a combination. Numerous large studies have shown that traditional combinations, consisting of an antipseudomonal β-lactam and an aminoglycoside, are not universally more powerful than monotherapy in the empiric treatment of uncomplicated episodes of fever in neutropenic patients. A third or fourth generation cephalosporin, a carbapenem, as well as piperacillin-tazobactam have been found to be effective single agents in the majority of cases.

It appears appropriate to reserve two-drug regimens for complicated cases or if antimicrobial resistance is a potential problem. The major disadvantages of an aminoglycoside are nephrotoxicity and ototoxicity, and the necessity to monitor serum levels. Combination of drugs such as amphotericin B, cyclosporine and cisplatin with an aminoglycoside is best avoided, because of their additive renal toxicity, whereas the high sodium contents may limit the simultaneous use of two β-lactam antibiotics in elderly patients. A large European Organization for Research and Treatment of Cancer – National Cancer Institute of Canada study showed unambiguously that vancomycin administered empirically for fever persistent despite appropriate initial antibiotic treatment can be withheld until the results of the cultures indicate the need for this antibiotic. Vancomycin must be included in an initial empiric regimen for patients known to be colonized with penicillin- and cephalosporin-resistant pneumococci and viridans streptococci or methicillin-resistant Staphylococcus aureus.

The choice to implement a particular antibiotic regimen is, at least partly, based on the results of clinical trials as reported in the literature. Yet, the results of such trials should be interpreted with great caution. Definitions for response as well as inclusion and exclusion criteria for clinical study protocols are usually very rigid and quite different from common clinical practice.
Table 2. Efficacy of antibacterial regimens in the treatment of neutropenic patients with fever

<table>
<thead>
<tr>
<th>Study</th>
<th>No of evaluable episodes (patients)</th>
<th>Treatment</th>
<th>Responses/ documented infections</th>
<th>Responses/ bacteraemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wade et al 1981</td>
<td>121(92)</td>
<td>Piperacillin + Amikacin</td>
<td>22/38 (58%)</td>
<td>5/15 (33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ticarcillin + Amikacin</td>
<td>19/34 (56%)</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Duprez, Michaux 1981</td>
<td>118</td>
<td>Piperacillin + Amikacin</td>
<td>26/34 (76%)</td>
<td>9/14 (64%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotaxime + Amikacin</td>
<td>27/37 (78%)</td>
<td>15/20 (75%)</td>
</tr>
<tr>
<td>Winston et al 1982</td>
<td>297 (244)</td>
<td>Piperacillin + Amikacin</td>
<td>38/53 (72%)</td>
<td>16/25 (64%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carbenicillin + Amikacin</td>
<td>48/66 (73%)</td>
<td>20/36 (56%)</td>
</tr>
<tr>
<td>Winston et al 1984</td>
<td>272 (219)</td>
<td>Piperacillin + Moxalactam</td>
<td>45/61 (74%)</td>
<td>17/23 (74%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moxalactam + Amikacin</td>
<td>41/50 (82%)</td>
<td>13/18 (72%)</td>
</tr>
<tr>
<td>Winston et al 1988</td>
<td>174(225)</td>
<td>Piperacillin + Cefoperozone</td>
<td>39/50 (78%)</td>
<td>22/29 (76%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piperacillin + Moxalactam</td>
<td>31/38 (82%)</td>
<td>16/22 (73%)</td>
</tr>
<tr>
<td>Sage et al 1988</td>
<td>170 (118)</td>
<td>Piperacillin + Netilmicin</td>
<td>12/15 (80%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ticarcillin + Netilmicin</td>
<td>11/14 (79%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mezlocillin + Netilmicin</td>
<td>11/18 (61%)</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefoperozone + Netilmicin</td>
<td>4/10 (40%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>De Pauw et al 1994</td>
<td>784 (696)</td>
<td>Ceftazidim</td>
<td>127/367 (35%)</td>
<td>33/118 (28%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piperacillin + Tobramycin</td>
<td>117/353 (33%)</td>
<td>25/132 (19%)</td>
</tr>
<tr>
<td>Cometta et al 1995</td>
<td>706 (475)</td>
<td>Piperacillin-Tazobactam + Amikacin</td>
<td>210/342 (61%)</td>
<td>40/50 (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidim + Amikacin</td>
<td>196/364 (54%)</td>
<td>35/101 (35%)</td>
</tr>
<tr>
<td>De Pauw et al 1995</td>
<td>304 (225)</td>
<td>Meropenem</td>
<td>54/110 (44%)</td>
<td>37/79 (47%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>35/105 (41%)</td>
<td>24/79 (30%)</td>
</tr>
<tr>
<td>Cometta et al 1996</td>
<td>483 (475)</td>
<td>Meropenem</td>
<td>270/483 (56%)</td>
<td>47/113 (42%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidim + Amikacin</td>
<td>245/475 (52%)</td>
<td>34/114 (30%)</td>
</tr>
<tr>
<td>Feld et al 2000</td>
<td>409 (471)</td>
<td>Meropenem</td>
<td>33/77 (54%)</td>
<td>14/31 (45%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>33/82 (44%)</td>
<td>22/43 (51%)</td>
</tr>
<tr>
<td>Del Favero et al 2001</td>
<td>733</td>
<td>Piperacillin-Tazobactam</td>
<td>67/186 (36%)</td>
<td>42/140 (30%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piperacillin-Tazobactam + Amikacin</td>
<td>60/176 (34%)</td>
<td>44/137 (32%)</td>
</tr>
<tr>
<td>Bow et al 2006</td>
<td>528</td>
<td>Piperacillin-Tazobactam</td>
<td>71/265 (27%)</td>
<td>11/81 (14%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftepime</td>
<td>54/263 (21%)</td>
<td>7/86 (8%)</td>
</tr>
</tbody>
</table>

Response is defined as resolution of fever and clinical signs of infection (where present) for four consecutive days, no relapse within 1 week of discontinuing therapy, and eradication of the infecting microorganisms (when isolated) without modification of treatment.
SPECIFICALLY TAILORED REGIMENS

Conduct of clinical trials in febrile neutropenic patients was a booming business in the mid-seventies and eighties when many new broad-spectrum antibiotics became available. The data derived from these trials have expanded our knowledge of the possible infectious complications tremendously. For instance, analysis of these studies revealed that only half of the patients who develop fever during neutropenia will present with a clinically or microbiologically documented infection, the majority being pulmonary infiltrates and bacteraemias (Table 3).49,50 Furthermore, it was obvious that neutropenic patients without a documented infection generally defervesced within a few days, whereas those with a clinically or microbiologically documented infection showed a much slower as well as an inferior response rate.19,27,51 This very consistent observation suggests that it might be prudent to select different antibiotic regimens for patients with different symptoms. Although there is no statistically valid evidence to support a more individually tailored approach, it appears reasonable to assume that patients might benefit from timely administration of the antibiotics with the highest intrinsic potency against a given microorganism. A focus of infection, if present, could help to select the most suitable additional anti-infective agents to compose a case-specific regimen because the location of an infection is, at least to a certain extent, indicative of specific infective agents (Table 4).52 Likewise, the results of surveillance cultures and knowledge of the common complications associated with particular anti-leukemic regimens may offer a valuable key to most appropriate initial treatment of a neutropenic patient with fever.

<table>
<thead>
<tr>
<th>Table 3. Classification of febrile neutropenic episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FUO- fever of unknown origin</strong></td>
</tr>
<tr>
<td><strong>Clinically documented infection</strong></td>
</tr>
<tr>
<td><strong>Microbiologically documented infection</strong></td>
</tr>
</tbody>
</table>
Table 4. Sites of infection and prevalent causative micro-organisms

<table>
<thead>
<tr>
<th>Site</th>
<th>Prevalent pathogens</th>
<th>Preferred antimicrobial agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper respiratory tract</td>
<td>Streptococci</td>
<td>amoxicillin, clindamycin</td>
</tr>
<tr>
<td>Lower respiratory tract</td>
<td>Gram-negative bacilli</td>
<td>combination therapy</td>
</tr>
<tr>
<td></td>
<td>Streptococci</td>
<td>amoxicillin, clindamycin, macrolides</td>
</tr>
<tr>
<td></td>
<td>Moulds</td>
<td>consider antifungal agents</td>
</tr>
<tr>
<td></td>
<td>Diffuse infiltrates (cytomegalovirus)</td>
<td>antiviral agents</td>
</tr>
<tr>
<td></td>
<td>Pneumocystis jiroveci</td>
<td>trimethoprim-sulfametoxazole</td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>Staphylococci</td>
<td>glycopeptides</td>
</tr>
<tr>
<td></td>
<td>Streptococci, anaerobes</td>
<td>amoxicillin, clindamycin, macrolides, glycopeptides</td>
</tr>
<tr>
<td>Abdominal</td>
<td>Anaerobes</td>
<td>metronidazole, glycopeptides</td>
</tr>
<tr>
<td>Perianal abscess</td>
<td>Gram-negative bacilli</td>
<td>combination therapy</td>
</tr>
</tbody>
</table>

GASTROINTESTINAL TRACT

A damaged integument probably plays a major etiologic role in virtually all infections that occur following aggressive cytoreductive therapy for a hematological malignancy but its involvement is most obvious in infections of the skin and GI tract. The use of high-dose cytarabine in conjunction with the occurrence of diarrhoea were found to be independent risk factors for streptococcal infections among 513 patients evaluated during the first episode of neutropenic fever.53 It has been recognized that bacteraemias due to oral *Streptococcus mitis* and *Streptococcus oralis* may bring serious complications such as a sepsis or an adult respiratory distress syndrome, which carry a high mortality.54-56 Similarly, bacteraemias due to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium* species as well as candidemias are more frequently encountered in patients with acute leukaemia who suffer from neutropenic enterocolitis or typhlitis, the ultimate disturbance of the delicate balance between mucosal barrier and microbial flora in the setting of prolonged exposure to antibiotics after cytotoxic therapy comprising intermediate or high-dose cytarabine chemotherapy.53 The signs and symptoms of chemotherapy-induced enterocolitis or typhlitis are very inconsistent and include nausea, vomiting, abdominal cramps and severe abdominal pain with virtually no bowel movements but accompanied by profuse, watery diarrhoea.7 Many patients are in such pain that they only gain relief from narcotic analgesics which, in turn, induce constipation by reduction of bowel movements. This may create a very alarming situation as the clinical picture of the severe variant resembles that of gut perforation or acute pancreatitis very closely. Because unnecessary surgical interventions in patients during
a hypoplastic bone marrow phase after treatment for an acute leukaemia can be detrimental, it is essential for physicians to be aware of the existence of this entity with the according symptoms. Ultrasonography or computer-assisted tomographic (CT)-scan, showing pathological thickening of the bowel walls, might be useful to establish the diagnosis of typhlitis. Patients treated for acute myeloid leukemia with a bowel wall thickness of more than 10 mm had a significantly higher mortality rate than did those with a bowel thickness of less than 10 mm. Disproportional bacterial overgrowth in the gastrointestinal tracts of patients with MBI can serve as a source of bacteraemia by the well-known gastrointestinal flora as well as by otherwise exclusively enteric pathogens such as *Clostridium septicum* and *Bacteroides fragilis*. In contrast, *Salmonella* species are rarely found in the stool or blood of granulocytopenic patients; these organisms are obviously not major players in this field; this is also true for pathogens like *Campylobacter* and *Shigella* species. Therefore, an adequate antibiotic regimen for patients with abdominal symptoms should cover for gram-negative rods but due consideration should be given to the use of compounds with activity against anaerobes. Next to glycopeptides and carbapenems, metronidazole appears an attractive adjunct to a standard regimen under these circumstances. Pseudomembranous colitis caused by *Clostridium difficile* constitutes a related but totally different entity that can be severe and even fatal. Stool should be tested immediately for *Clostridium difficile* toxin if the diagnosis is suspected. Enteric *Clostridia* infections necessitate oral antibiotic therapy with either vancomycin or metronidazole. Relapses are frequent and may follow cancer chemotherapy or courses with antibiotics such as clindamycin. Relapse is harder to document because toxin may persist in the stool of successfully treated patients.

Diagnostic problems are held accountable for underestimating enteric viruses as causative agents in gastrointestinal infections. Although a compromised cell-mediated immunity is known to predispose for parasitic and protozoan infections, their incidence is surprisingly low in patients who are treated for a hematological malignancy.

**SKIN**

Folliculitis and cellulitis are the most common manifestations of infectious processes in the skin. Sometimes it is difficult to differentiate infectious lesions from drug-induced toxic skin eruptions. Infection-associated erythema and swelling are usually mild but, if left untreated, infiltration and abscess formation will involve extensive areas of the skin with necrosis and gangrene. Since the lesions associated with the various organisms are rather alike, a simple needle aspiration or biopsy should be performed to establish an accurate diagnosis as early as possible in the course of the disease. The causative micro-organisms include streptococci, staphylococci, and, less commonly, gram-negative bacilli and fungi. Localized infections of the skin, particularly in the face, are usually caused by gram-positive bacteria that preferably arise in carriers of organisms like *Staphylococcus aureus*. None of the standard empiric regimens is the optimal choice for treating infections by the prevalent but usually indolent...
non- *Staphylococcus aureus* gram-positive cocci that are often methicillin-resistant, but the morbidity from these infections should not be underestimated either. *Pseudomonas aeruginosa* acquired in a hot Jacuzzzi may cause a folliculitis that occasionally progresses to a destructive ecthyma gangrenosum.\textsuperscript{71} This characteristic entity should be distinguished from similar lesions caused by other rare pathogens, such as actinomyces, *Stenotrophomonas maltophilia*\textsuperscript{68} and fungi\textsuperscript{69,70} as well as from pyoderma gangrenosum, a noninfectious cutaneous process in patients with a myeloid malignancy.\textsuperscript{72,73} Sweet’s syndrome, a dense, tender infiltration by neutrophils of the dermis on the head, neck, and upper extremities is associated with a leukocytosis.\textsuperscript{74} Varicella zoster is the leading dermatologic complication in patients with an impaired cell-mediated immunity.\textsuperscript{75-77} If skin or mucous membrane lesions due to *Herpes simplex* or *Varicella zoster* viruses are present, even if they are not the cause of fever, treatment with valaciclovir is indicated in the intention to enhance the healing of lesions that could become potential portals of entry for bacteria and fungi.\textsuperscript{22} The results of several prospective studies do not indicate a general need for a glycopeptide as part of the front-line therapeutic regimen unless one has a particular reason to suspect the presence of methicillin-resistant *Staphylococcus aureus* on the basis of local patterns of resistance. Nevertheless, most physicians intuitively prefer an up-front glycopeptide-containing regimen for apparently catheter-related infections as these are frequently due to coagulase-negative staphylococci (CoNS), although early glycopeptides barely contribute to the chance of survival from these usually indolent infections. Hence, when CoNS are involved, a few days of watchful waiting for a possible clinical response and the results of the cultures will have no detrimental impact. Most catheter-associated infections will respond to antibiotic therapy without removal of the catheter. Rotation of antibiotics through each lumen of multilumen catheters to avoid microbial sequestration in one of the lines and the use of antibiotic-containing heparin lock solutions to supplement systemic therapy have been proposed by some investigators but such practices remain controversial.\textsuperscript{22} Pulling the catheter is most likely to be required for cure if a concurrent venous thrombosis is found, the tunnel tract appears involved or if the infection, regardless of the etiology, is recurrent or if after several days of therapy the response to antibiotics appears doubtful.\textsuperscript{78}

**UPPER RESPIRATORY TRACT**

Gingivostomatitsis and periodontal lesions occur frequently in patients with acute leukaemia.\textsuperscript{79} Oral MBI is characterized by pain, edema, erythema, superficial lesions, pseudomembranous formation in conjunction with excessive mucous production, reduced saliva secretion and bleeding. A wide array of pathogens can be found and include *Herpes simplex*, gram-negative bacilli, streptococci, anaerobes, and *Candida* species.\textsuperscript{80} With the introduction of aggressive chemotherapeutic regimens, hitherto unusual pathogens such as *Stomatococcus* and *Aerococcus* are increasingly seen in patients with oral MBI. Mixed and polymicrobial infections are more or less standard.\textsuperscript{80,81} Given the range of prevalent pathogens there is little need to deviate from one of the standard regimens, although on theoretical grounds one might prefer to select a
Managing fever during neutropenia

Carbapenem or extended-spectrum penicillin given their superior intrinsic activity against viridans streptococci and pneumococci. The course of Herpes simplex stomatitis is usually protracted in patients treated for leukaemia or lymphoma, and relapses are common. Herpes simplex lesions are most commonly white painful plaques on the gums, tongue, buccal mucosa, or oropharynx and may be difficult to discriminate from oropharyngeal candidiasis and, indeed, co-infections do occur. Swallowing can be so painful that saliva is expectorated and intake of food and fluids drastically reduced. It is not uncommon for oropharyngeal Herpes simplex and Candida infection to extend to the oesophagus. Although neither herpes nor candidiasis do belong to the category of diseases that requires an empiric approach, it is generally contended that early treatment with valaciclovir and fluconazole, respectively, is important to prevent extension into the oesophagus and further dissemination, particularly among bone marrow transplant recipients. When the paranasal sinuses are involved in the infectious process, moulds have to be considered as possible causes. As the clinical picture has typical features, direct inspection of the nasal turbinates and a CT-scan of the sinuses can be helpful to establish or reject the diagnosis.

LOWER RESPIRATORY TRACT
Management of pulmonary infiltrates that are liable for 70% of all fatal infections in febrile neutropenic patients is complex. The classic clinical complaints of cough, pain and dyspnoea should not be neglected but bronchoscopy and radiological examination of the chest by a CT-scan represent the cornerstones of all diagnostic procedures. Typically, chest radiographs performed early in the evolution of infection in patients with profound granulocytopenia fail to show infiltrates. It may take more than 3 days for the infection to generate enough necrosis with haemorrhage and oedema to produce a visible infiltrate. The critical decision faced by the clinician at the bedside of patients with pulmonary infiltrates is whether to undertake invasive procedures such as bronchoscopy with or without bronchoalveolar lavage, transbronchial biopsy, transthoracic aspiration, thoracoscopy-guided biopsy, or open lung biopsy. The exact role of these diagnostic procedures in the optimal management of patients is still controversial because the yield depends on the collaboration and skills of various specialists. Moreover, concurrent thrombocytopenia precludes simple invasive diagnostic procedures in many patients. The radiologic pattern of a possible infiltrate is often suggestive of its cause. A diffuse opacity, usually of both lungs, is seldom of bacterial or fungal origin. Although viruses and Pneumocystis jiroveci typically cause diffuse, bilateral pulmonary infiltrations, it should be kept in mind that a similar picture of pneumonitis can be seen secondary to radiation, fluid overload, cytotoxic drugs such as methotrexate, cytarabine and bleomycin, and in pulmonary haemorrhage. Pneumocystis jiroveci pneumonia is manifested in patients with deficient cellular immunity as fever, progressive hypoxemia with dry cough, and dyspnoea, typically beginning after discontinuation of corticosteroid therapy given for other reasons. High-dose trimethoprim-sulfamethoxazole with adjuvant corticosteroids for hypoxemic patients (PO₂ < 70 mmHg) has become the preferred
therapy for these infections. Alternatives include intravenous pentamidine, oral dapsone in combination with trimethoprim, or oral atovaquone suspension alone. Antiviral drugs are indicated only if there is clinical or laboratory evidence of viral disease. With the exception of a cytomegalovirus related pneumonitis in allogeneic bone marrow transplant recipients with graft-versus-host disease, there appears to be no need for empiric cover of respiratory viruses, such as respiratory syncytial virus, influenza and adenoviruses. Ganciclovir, valganciclovir and foscarnet have established activity in the treatment of cytomegalovirus infection and their timely use might be life-saving. *Mycoplasma pneumoniae* with or without cold agglutinins is remarkably infrequent in patients treated for leukaemia. In more acutely ill patients, the possibility of acute lung injury following transfusion of a cellular blood product or a respiratory distress syndrome related to streptococcal sepsis should be considered. Particularly patients with an infection by *Streptococcus mitis*, which has been linked with severe MBI and high-dose cytarabine are at risk. The incidence of an acute respiratory distress syndrome in such cases is more than 20 percent and the mortality is substantial. The pathophysiology of an adult respiratory distress syndrome following streptococcal bacteraemia in a neutropenic patient is poorly understood. Probably several factors are involved, such as a deleterious effect of microorganisms superimposed on preexisting tissue damage. Even patients who had received the appropriate antimicrobials from the onset of fever were reported to experience shock and death. Therefore, next to supplementary antibiotics, corticosteroids should be considered in the management of patients affected by this complication. Bacterial infections of the lung, accompanied by a bacteraemia in about 50 percent of cases, usually create shadows on a CT-scan that are confined to one or more lobes. Pneumonias caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* do have a bad reputation but enterobacteriaceae, Haemophilus influenzae and Streptococcus species are hardly less dangerous. Given the uniformly poor outcome of pulmonary infections in clinical trials, the empiric use of a combination of antibiotics is recommended with the addition of vancomycin in centers that face resistance of Streptococcus pneumoniae to penicillin and macrolides. Outbreaks of Legionella pneumophila, an infection characterized by patchy interstitial or nodular pulmonary infiltrates and sometimes accompanied by headache or gastrointestinal symptoms, have been observed among compromised patients in units with contaminated water systems. Therefore, if a case of legionellosis is encountered other patients with similar symptoms on the same ward should be protected with a macrolide or a fluoroquinolone from the start of antimicrobial therapy. Conversely, a nodular pattern of pulmonary infiltrates should urge the physician to consider the possibility of atypical pneumonia or, more commonly, a pulmonary fungal infection but for such cases diagnostic procedures rather than immediate institution of antifungal drugs should be given priority. Especially in patients with a concomitant impairment of the cell-mediated immunity, pulmonary aspergillosis has to be distinguished from tuberculosis. Infections with *Mycobacterium tuberculosis* in patients with impaired cell-mediated immunity are manifested as either localized pulmonary disease or devastating miliary tuberculosis. Nontuberculous mycobacteria are still rather rare in patients
with acute leukaemia, but the introduction of purine analogues such as cladribine and fludarabine, which cause severe and prolonged depression of cellular immunity, may change this picture in the near future.92

OTHER FOCI OF INFECTION
Urinary tract infections are astonishingly uncommon in patients who are treated for a leukaemia or lymphoma and since gram-negative bacteria are the predominant pathogens the choice for a single broad-spectrum β-lactam is fully justified.12 Malignant otitis externa is very serious infectious complication that can emergence after the administration of aggressive chemotherapy for a hematological malignancy.93 At the outset the patient will complain of a painful, discharging ear, whereas physical examination will reveal a vulnerable oedematous ear canal. Local maceration and humid conditions favour the growth of Pseudomonas aeruginosa which, indeed, can be isolated frequently from swabs taken from the superficial lesions. Subsequently, the infection will penetrate into underlying soft tissues, filling the retromandibular and parotid area. Likewise, spreading to the middle ear, the mastoid air cells and adjacent temporal bone is possible. Once osteomyelitis has been established extension to the basic of the skull with invasion of the cranial nerves and local thrombosis poses a direct danger to the patient’s life. A CT-scan may be helpful to discover tissue damage in an early phase. Prolonged antibiotic therapy with ceftazidime or ciprofloxacin in combination with surgical debridement constitutes the treatment of choice.93 Occasionally, a similar clinical picture can be the result of an infection by Staphylococcus aureus or Aspergillus fumigatus. In such cases surgery should be combined with flucloxacillin or vancomycin and voriconazole, respectively. An insidious onset of fever accompanied by headache and confusion might be indicative of meningitis when localization of the leukaemia or lymphoma has been excluded by cytologic examination of the cerebrospinal fluid. In case of infection, the cerebrospinal fluid is usually clear with a moderate protein elevation. The prevalent pathogens are Listeria monocytogenes, Cryptococcus neoformans and Toxoplasma gondii.65 Recovery of one of Listeria monocytogenes and Cryptococcus neoformans from blood cultures should, provided that no intracranial hypertension is detected, always prompt a lumbar puncture even in the absence of neurological symptoms. Considering their low incidence and the relatively reliable diagnostic possibilities, there is no need to provide cover for these infections with a specifically adapted empiric regimen.

MANAGEMENT ON AN OUT-PATIENT BASIS
When potent oral broad-spectrum antibiotics became available in the late eighties many a clinician felt tempted to use these drugs in the treatment of febrile neutropenic patients. Several groups around the world took the responsibility to assess the options and limitations of this seemingly revolutionary approach systematically.95-97 These analyses showed that it is possible
to define risk factors that can be used to classify patients into low or high-risk categories. In fact, these studies offered nothing more than identification of objective parameters that corroborate the gut’s feeling of the experienced clinician in an era of litigation. Since the time of Bodey, it was already obvious that patients with absolute neutrophil count between 0.1 and $0.5 \times 10^9/l$ (100-500/ml) carry a minor risk when compared to those with a count of less than $0.1 \times 10^9/l$ (100/ml) but now other factors have been added.¹ Patients with concurrent mucosal damage or impaired cellular immunity, as well as those with clinically documented infections or dubious vital signs are at high risk deserve optimal vigilance and cannot be considered candidates for antibiotic treatment on an out-patient basis. The vast majority of patients under treatment for an acute leukemia are considered high-risk patients and should continue to receive intravenous broad-spectrum antibiotics. The remaining low-risk patients, namely those with unexplained fever who are clinically stable, may be safely treated with oral antibiotics provided that they have been seen at a qualified medical center promptly after the onset of fever.⁹⁵,⁹⁶ The possible use of antibiotic prophylaxis does not pre-empt the need for a thorough check-up but limits the choice of drugs that can be used for treatment. Patients with increasing granulocyte counts are considered to be better candidates for outpatient therapy than are patients without an indication of bone marrow recovery. Among the oral regimens that have been evaluated are ofloxacin, ciprofloxacin, and ciprofloxacin plus amoxicillin-clavulanate. It is crucial to make sure that the patient is informed about the risk of unremitting fever during a neutropenic episode and that he or she fully understands the importance of seeking immediate medical advice in case any unexpected incident occurs. Vigilant observation at home by a relative or professional health care worker and prompt access to appropriate medical care must be assured 24 h per day, seven days a week.⁹⁸-¹⁰¹ As an alternative to initial outpatient therapy, early discharge with continued outpatient therapy for selected patients may be considered after a brief admission during which intravenous therapy is initiated, fulminant infection is excluded, and appropriate culture specimens are taken.¹⁰²,¹⁰³ Two studies have demonstrated that children who lack signs of sepsis and severe MBI, who are afebrile for >48 h, who have neutrophil counts of >100 cells/mm³ (>0.5 x 10⁹/l), and who are at low risk for complications may have their intravenous antibiotic treatment safely stopped to be substituted by oral cefixime.¹⁰⁴,¹⁰⁵
MANAGEMENT OF FEVER AFTER THE EMPIRIC EPISODE

PRINCIPLES
After starting empiric treatment, fever will persist or return in about one third of patients. The average duration of fever in serious infections, in eventually successfully treated neutropenic patients is 4-5 days (Table 2, Figure 1).\textsuperscript{4,9,13,14,34-44}

![Figure 1. Typical course of fever after institution of empiric antimicrobial therapy in neutropenic patients\textsuperscript{4}](image)

Although fever can be inconvenient for the patient, it is important to realize that it is part of the body’s defence system.\textsuperscript{106} Indeed, some retrospective studies have suggested that fever is associated with improved survival and shortened disease. Uncontrolled studies have reported an association of increased mortality with the absence of fever in polymicrobial or gram-negative sepsis and in elderly patients with community acquired pneumonia.\textsuperscript{107,108} So, when the body temperature remains above normal during three or four days on apparently effective broad-spectrum antibiotics, this should not be considered a complete waste of time, particularly not if the time is used for an appropriate diagnostic work-up. It should be kept in mind that empiric administration of antibiotics is only meant as an immediate cover for rapidly fatal bacteria
such as gram-negative rods and *Staphylococcus aureus*, thereby, so to say, buying time for consideration of the next therapeutic interventions and for waiting for the results of the diagnostic procedures. When the results of the cultures have become available and the infection has had the time to blossom clinically, there is a more solid basis for decisions on necessary adjustments of an antibiotic regimen.

Unfortunately, all large, randomized clinical trials on empiric antibiotic therapy in the febrile neutropenic patients during the past 30 years have been pharmaceutical company-driven for purposes of attaining governmental agency approval.\textsuperscript{23,24} By consequence, the design of these studies focused primarily on the efficacy of a particular drug in comparison with another drug or a combination of drugs. According to the protocols for these trials, only patients who survived the episode without a change in the allocated regimen could be labeled as successes, whereas any change in therapy, independent of the trigger, had to be denoted a failure. Therefore, modification of the test regimens was discouraged, which constitutes a rather artificial situation, as clinicians are inclined to adjust an antibiotic regimen for no other reason than a subjective feeling of dissatisfaction with the current antibiotics. Changes often reflect the lack of confidence on the part of the clinician rather than any deficiency in the regimen used. When restrictions surrounding a clinical trial do not apply, juggling antibiotics against an undulating line on a temperature chart is a well-known happening on a ward with patients suffering from a hematological malignancy care. Indeed, in daily practice many modifications are not based on objective criteria and are made outside office hours, \textit{i.e.} by often less experienced physicians on call.\textsuperscript{103,109} However, it is generally recognized that exposure to many different antibiotics as a result of haphazard changes of regimens enhances the risk of drug-related adverse and seldom improves the outlook for the patient under treatment. Moreover, such a policy of unmotivated changes of antibiotics might wrongly decrease the perceived need for further diagnostic procedures in poorly responding patients. Since there is evidence from clinical trials on what to do after the empiric phase, some experts have been propagating so-called algorithms of planned progressive antibiotic therapy to treat neutropenic patients with fever. A planned progressive strategy involves adjustment of therapy every two to three days, until the patient becomes febrile or until all the potential causes of infection are covered by the best available microbial agents, irrespective of the development of additional symptoms. It is clear that algorithms featuring planned progressive therapy are destined to lead to over-treatment with unnecessary expenses and drug exposure.\textsuperscript{108} It appears more intellectually attractive not to rely on fixed algorithms but to weigh several different, patient-specific parameters, including fever and the clinical response, as a guidance for modification of an empiric regimen. It goes without saying that spending time at the bedside is crucial for those who feel attracted by the role of attending physician because careful observation often provides early clinical clues for a rational adaptation of the actual regimen. The need for individuality is not only dictated by variations in the signs and symptoms of the patient that accompany persisting fever but also by the differences in skills and expertise amongst the attending specialists in the various centers.
For example, centers with excellent and interested departments of medical microbiology will rely more heavily on their findings than do centers with a poorly functioning facility, whereas units with an active radiology may benefit from the locally available know-how in this particular field.

**MODIFICATIONS IN POORLY RESPONDING PATIENTS**

**CASE-BY-CASE MODIFICATION OF AN INITIAL EMPIRIC REGIMEN**

Once an empiric antibiotic therapy has started the patient must be monitored continuously for non-response, emergence of secondary infections, adverse effects, and the development of drug-resistant organisms. This implies that the start of antibacterial agents cannot be seen as an impetus to stop diagnostic procedures. Daily blood cultures are certainly justified as long as patients remain febrile and when a new temperature peak occurs because breakthrough bacteraemia or fungaemia may develop. Close monitoring of sites that are prone to infection should start before the onset of fever and has to be continued after empirical antibacterial therapy has commenced. Subtle changes must bring diagnostic tools into play to confirm or exclude the presence of an infectious focus. Regular CT-scans of the chest, preferably in combination with serological monitoring for *Aspergillosis* antigen, have an established value in patients who are at increased risk of fungal infections.

As a rule, approximately 65 percent of patients without a focus of infection, of which 30 percent with positive blood cultures, will have shown some clinical improvement after three days of broad-spectrum empiric cover in spite of persisting fever in a substantial number. In most cases defervescence will follow rapidly. Elements that should be incorporated in clinical decision making include the course of fever and clinical condition with special attention for the vital signs, evolving symptoms of infection in relation to the granulocyte count, CRP levels, presence of antigens, and antiviral titers. The results of all cultures taken at the onset of fever have to be assessed and it is recommended to analyse surveillance cultures, if any, to identify possibly colonizing resistant organisms. Without clinical deterioration or proof of an infection caused by a micro-organism resistant to the allocated antibiotic regimen, persisting fever after 72 to 96 hours of empiric therapy is an unsatisfactory basis for changing the actual antibacterial regimen. It is better to alter the regimen only when there are objective reasons to do so: deterioration of vital signs, isolation of a resistant pathogen without clinical improvement, persistence of a pathogen, antibiotic-related adverse events, occurrence of a new focus of infection or progression of an existing focus in the absence of granulocyte recovery, unexplained fever persisting for more than 5 days, new fever, a new pathogen or recognition of a local epidemic with a resistant organism. In most patients antimicrobial therapy can be adjusted objectively on the basis of clinical or microbiologic findings but such an individually
tailored approach requires careful daily assessment of all possible parameters involving all consulting specialists, including microbiologists, pulmonologists and radiologists. In contrast to the moment of the onset of fever there is ample time for deliberation and contemplation in a situation where the patient’s fever persists for three or more days while on antibiotics because the origin of fever is obviously not a rapidly fatal micro-organism that needs immediate cover. Fever that persists for more than three days suggests that the patient has a nonbacterial infection, a resistant bacterial infection, a second infection, or a non-infectious cause of fever like drug fever (Table 5).

Table 5. Possible causes for lack of response to antibiotics

- a bacterial infection resistant to the antibiotics
- cell wall-deficient bacteraemia
- infections at a avascular site (e.g., abscesses or catheters)
- inadequate serum and tissue levels of the antibiotics
- slow response to the drugs in use
- the emergence of a second infection
- a nonbacterial infection
  - fungal
  - viral
  - post-transplant lymphoproliferative disease
  - parasitic
- pyrogenic substances
  - cytokines
  - (auto)immune reactions
  - blood product antigens
  - toxins
  - drugs (antibiotics!)
  - tissue (tumor) products

Despite extensive cultures, only around 30 percent of all febrile patients will be shown to have microbiologically defined infections. In 30 percent of patients organ involvement is already obvious when fever arises and an additional 10 percent will show a clinically defined infection within next 72 hours (Figure 2).
Managing fever during neutropenia

Others have neither a focus of infection, nor a positive culture and are defined as unexplained fevers. Using clinical well-being as a leading parameter, there are roughly three possible situations after three days of treatment: the patient’s condition is a) improving (approximately 55 percent of cases); b) stable (approximately 35 percent); or c) has deteriorated (10 percent) (Table 6). Patients belonging to each of these three categories can suffer from either a microbiologically documented infection, a clinically documented infection or unexplained fever. All these factors that are partly subjective and partly objective can be exploited to steer the modification of an empiric regimen whenever there is a perceived need to do so. Ultimately, only 15 to 20 percent of patients with a persisting unexplained fever would require a continued empiric approach after 72 hours of broad-spectrum antibacterial therapy. Whichever modification is planned, it cannot be overemphasized that maintenance of appropriate anti-gram-negative cover is mandatory as long as a patient is febrile and neutropenic.22
Table 6. Considerations for modification of antibiotic regimens in febrile neutropenic patients

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Microbiologically Documented Infection</th>
<th>Clinically Documented Infection</th>
<th>Unexplained Fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improving Clinical Condition</td>
<td>• Maintain gram-negative cover</td>
<td>• Adjust on the basis of susceptibility pattern</td>
<td>• Consider switch to an oral regimen</td>
</tr>
<tr>
<td>Stable Clinical Condition</td>
<td>• Maintain gram-negative cover</td>
<td>• Continue running regimen</td>
<td>• Consider adaptation on the basis of focus-specific pathogens</td>
</tr>
<tr>
<td>Deteriorating Clinical Condition</td>
<td>• Maintain gram-negative cover at maximally tolerated doses</td>
<td>• Maximal cover of focus-prevalent pathogens</td>
<td>• Consider further diagnostic procedures</td>
</tr>
<tr>
<td></td>
<td>• Consider adjustment on the basis of susceptibility pattern</td>
<td></td>
<td>• Consider institution of intravenous antifungals</td>
</tr>
</tbody>
</table>
MICROBIOLOGICALLY DOCUMENTED INFECTIONS

When the patients is improving or stable there appears to be no imminent need to adjust a running regimen. Depending on the micro-organism isolated, a change to an oral regimen could be considered with caution. In case a gram-negative isolate is identified, broad-spectrum antibiotic coverage should be maintained in full dose. If, besides a gram-negative isolate, another pathogen is found a specific agent should be added. Whereas the clinical relevance of a blood culture positive for gram-negative bacilli is never a matter of controversy, the implication of recovery of particular gram-positive cocci is questionable. Single blood cultures positive for Staphylococcus aureus, Streptococcus pneumoniae, or Enterococcus faecalis in neutropenic patients should be regarded as significant and indicative for the choice of further treatment. Viridans group streptococci, with an average mortality of 15 to 20 percent are perhaps the most feared among the bacteraemias today. Although viridans streptococci are common blood contaminants in the general population, positive blood cultures in patients with MBI should not be disregarded, certainly not when it concerns Streptococcus mitis. Isolation of rare micro-organisms should prompt to evaluation of the appropriateness of the starting antibiotic regimen, when the patient is not responding optimally. On the other hand, isolation of in vitro resistant organisms such as CoNS and, more rarely, Stenotrophomonas maltophilia, from the blood of a clinically evidently improving patient poses an interesting challenge. Many would be inclined to modify the actual regimen but there is no evidence that this tactic is beneficial as other bacteria that were not grown on the culture plate could have been the culprits in the current febrile episode. A blood culture that yields Candida species or another fungus should be taken very seriously and dictates immediate institution of antifungal therapy. The availability of the candins has extended the therapeutic options. Adaptations of an antibiotic regimen in a patient who is clearly not responding is straightforward when a micro-organism has been isolated; the results of the cultures, supplemented by susceptibility testing, will assist in selecting the proper antibiotics.

CLINICALLY DOCUMENTED INFECTIONS

All clinical trials so far have demonstrated consistently that patients diagnosed with a clinically documented infection respond much slower and remain febrile for a longer time than those without a focus of infection. Moreover, due to problematic penetration into avascular sites infections associated with abscesses or prosthetic devices usually respond poorly to antimicrobial therapy. Attending physicians should, therefore, be more hesitant to change antibiotics in patients who are not deteriorating. On the other hand, there are indications that early addition of specific agents might be useful for a more rapid control of the infection. For instance, considering the probable involvement of anaerobes, switching to a carbapenem, if not given initially, or addition of metronidazole to a standard anti-gram-negative regimen appears a logical choice when fever is accompanied by abdominal symptoms. In cases with a clinically documented site, who do not improve or stabilize, coverage of micro-organisms known to
prevail at the involved site of infection (Table 4) appears appropriate. Clinically documented infections that emerge later during the course of febrile neutropenia carry a dismal prognosis and is presumed to be related to the occurrence of resistant micro-organisms, including invasive fungi, in combination with a persisting immunodeficiency as a result of a refractory underlying disease.\textsuperscript{20,69}

**PERSISTENT UNEXPLAINED FEVER OR FEVER OF UNKNOWN ORIGIN**

If the patient with an unexplained fever clinically improves or remains stable after 72 hour of empirical treatment and re-evaluation by physical examination and diagnostic tests yields no new information, and no isolate was found, the initial antibiotic regimen can be continued or can be switched to an oral compound. The latter decision will be strengthened if the neutropenia can be expected to resolve within the ensuing days. If vancomycin is a component of the initial antimicrobial regimen, withdrawal of the drug should be considered if the results of the cultures do not support its use.\textsuperscript{22}

Deteriorating cases without any microbiological or clinical sign of infection pose a dilemma. Unexplained fever accompanied by deterioration can imply that the patient has a non-bacterial infection or a non-infectious cause of fever, but foremost a resistant bacterial infection or the emergence of a second infection should be taken into account.\textsuperscript{19,110} An initial response rate of about 35 percent may be expected in patients with shock, as compared with 70 percent in patients without shock, which suggests the presence of an undetected toxin-producing pathogen. A fortification of the actual antibacterial regimen is mandatory in critically ill patients, independent of the level of fever.\textsuperscript{106} Escalation might include filling theoretical gaps in antibiotic cover and enhanced monitoring for any change in the patient’s condition. Under these circumstances, the selection of agents should be guided by knowledge of locally prevalent virulent pathogens and the actual susceptibility pattern, which implies the necessity of a close cooperation with local microbiologists. Addition of vancomycin appears logical in view of the relatively low yield of blood cultures and the fact that the spectrum of antibacterial drugs in traditional empiric regimens usually does not cover for coagulase-negative staphylococci, methicillin-resistant \textit{Staphylococcus aureus}, enterococci, and some strains of penicillin-resistant \textit{Streptococcus pneumoniae} and viridans streptococci.\textsuperscript{11} On the other hand, liberal use of vancomycin has confronted the medical community with vancomycin-resistant enterococci and staphylococci, which led to the introduction of new agents like quinupristin-dalfopristin and linezolid in the treatment of febrile neutropenic patients.\textsuperscript{22} When the starting regimen has consisted of a single, broad-spectrum \(\beta\)-lactam, the addition of an aminoglycoside seems to be an attractive option to provide a better protection against infections by resistant gram-negative rods.\textsuperscript{46,47} However, it has to be emphasized that development of resistance during therapy is extremely rare and that aggressive gram-negative organisms typically push the infection in a stage beyond cure within a few days in most cases. Hence, if the local resistance pattern or a particular concern in an individual patient prompts the use of an aminoglycoside...
it should be prescribed from the start in optimal doses with monitoring of the peak and serum levels.\textsuperscript{46,47} Besides, clinical deterioration in a persistently neutropenic patient with unexplained fever is an important but rather rare event in daily practice and applies to only a quarter of the 10 percent of cases that show deterioration while on broad-spectrum antibacterial treatment. Moreover, it is noteworthy that the success rate of empiric modifications is less than 20 percent, whereas more than 50 percent of cases will respond to specifically customized modifications.\textsuperscript{41}

**SPECIFIC CONSIDERATIONS**

**INVASIVE FUNGAL DISEASE**

Invasive fungal infections are encountered in up to 40 percent of autopsies in patients with hematological malignancies.\textsuperscript{20} Fungi have been found to be responsible for two thirds of all super-infections, which surface during broad-spectrum antibiotic treatment of neutropenic patients. More than 20 years ago, when diagnostic facilities were virtually non-existent and the choice of effective antifungal agents limited, two prospective, randomized trials laid the scientific foundation for the addition of systemically active antifungals to a purportedly insufficient antibacterial regimen.\textsuperscript{111,112} This strategy appeared to reduce the incidence of invasive fungal infections in patients without any further sign of a clinically documented infection. Solid statistical evidence to support the validity of this empiric approach was never obtained in further statistically valid, placebo-controlled trials. This so-called empiric antifungal therapy has remained popular as it seemed to make life easy for clinicians. The lack of reliable diagnostic tools in combination with a very poor outcome of invasive fungal infections that were not timely treated contributed greatly to this popularity.\textsuperscript{117,118} However, in most cases empirically given antifungals are redundant because there is simply no invasive fungal infection at all. A better understanding of the pathophysiology of invasive fungal disease in combination with better diagnostics allows for a more differentiated approach.\textsuperscript{117-120} An optimal diagnostic work-up in conjunction with careful clinical observation will likely render routine empiric antifungal therapy superfluous in most cases because appropriate application of presently available diagnostic tools enables timely pre-emptive institution of appropriate antifungal therapy by experienced clinicians.\textsuperscript{121-123} The most common initial presentation of invasive aspergillosis is unremitting fever despite broad-spectrum antibacterial treatment, eventually accompanied in most patients by pulmonary infiltrates or sinusitis. Clinicians should suspect the diagnosis in a patient with pleuritic pain, hemoptysis, and a localized rub. The halo sign (a dense central nodule with surrounding less dense infiltrate) on a CT-scan of the chest, though not pathognomonic, is highly suggestive of an early phase of pulmonary aspergillosis or other mould pneumonia in immunosuppressed patients.\textsuperscript{124-126} Even when gram-negative pathogens, including *Pseudomonas aeruginosa* and *Enterobacter cloacae*, are concomitantly isolated from the sputum or blood of such patients, aspergillosis should be the leading consideration. If no infiltrate is found in a
high-risk patient with persisting fever, the investigation has to be repeated within a few days, preferably supported by a bronchoalveolar lavage and additional assays such as screening for the presence of galactomannan in the blood.\textsuperscript{121} Even in adequately responding patients the CT-scan will usually show some enhancement of the lesion when the neutrophils return with, eventually, development of a cavitation within the infiltrate, the so-called air-crescent sign.\textsuperscript{124-126} This finding is suggestive of aspergillosis, although mucormycosis and other agents may cause an identical picture. Whether the increased incidence of non \textit{Aspergillus} mould is due to more extensive use of new azoles like voriconazole or to the use of more immunosuppressive treatment schemes remains to be seen.\textsuperscript{127,128} Isolation of an \textit{Aspergillus} species from sputum or bronchoalveolar lavage specimens connotes either invasive infection or bronchial colonization, the latter conferring high risk for invasive aspergillosis. When voriconazole or posaconazole have been used as prophylaxis, it is sensible to select an antifungal compound with a different mode of action when therapy becomes mandatory.\textsuperscript{129,130} Surgery is indicated for patients in whom lesions near the pulmonary hilus pose a threat of invasion of a major vessel with the risk of fatal haemorrhage or for debridement of dead tissue after a period of antifungal therapy.\textsuperscript{126} Low risk patients who test negative for \textit{Aspergillus} in all diagnostic procedures do not need to be started on intravenous antifungals. Treatment should be stopped for those patients started on antifungals pending diagnostic test results. A more conservative wait-and-see approach can be implemented successfully once clinicians learn to accept that negative diagnostic results constitute sufficient evidence that there is no fungal infection in many persistently febrile neutropenic patients.\textsuperscript{121,123} Fluconazole given as prophylaxis has virtually eliminated infections with \textit{Candida albicans}. However, \textit{Candida} species or other fungi are still occasionally identified as causes of disseminated infections in humans, albeit that there has been a shift from \textit{Candida albicans} to non-albicans species.\textsuperscript{131,132} A candidemic patient typically presents with an irregular fever sometimes accompanied by polymyalgia and polyarthralgia. In about 10 percent of cases characteristic pinkish-purple, nontender subcutaneous nodules may arise anywhere on the body. Biopsy specimens should be cultured and histologically screened at multiple levels in an attempt to establish a final diagnosis. \textit{Candida} ophthalmitis is seldom seen in leukemic patients since the distinctive retinal exudates are the result of an inflammatory response that involves granulocytes. Upon return of the neutrophils or tapering of corticosteroids, complaints of abdominal discomfort and elevation of alkaline phosphatase levels with or without hepatosplenomegaly may emerge. At this stage an abdominal ultrasound or CT-scan will display rather distinctive multiple abscesses in the liver and/or spleen, known as “bull’s eyes”.\textsuperscript{133,134} The mortality of an invasive yeast infection may be as high as 40 percent, particularly when the start of antifungal therapy has been postponed. Trichosporonosis and fusariosis can produce a clinical syndrome identical to candidemia.\textsuperscript{135-137}
BIOLOGICAL RESPONSE MODIFIERS

Up to now empirical antimicrobial therapy has been the backbone of improving survival of febrile neutropenia in leukemic patients. Hematopoietic growth factors have been studied as adjunctive therapy for febrile neutropenic patients in several randomized, controlled trials. Granulocyte colony stimulating factor (G-CSF; filgrastim) and granulocyte-macrophage colony-stimulating factor (sargramostim) when used as part of the treatment of febrile neutropenic patients were shown to consistently shorten the duration of neutropenia defined as a neutrophil count below $0.5 \times 10^9/l$ (500/ml). However, the duration of absolute neutropenia, i.e. count of less than $0.1 \times 10^9/l$ (100/ml), was not influenced, which might help to explain why neither a decrease in infection-related mortality rates nor a significant effect on morbidity, including duration of fever and use of anti-infectives, were observed. Therefore, the use of growth factors should be restricted to complicated cases for which there appears to be no rational alternative therapeutic option. This concept also applies to the use of granulocyte transfusions. Transfusion of high numbers of granulocytes harvested after administration of G-CSF, with or without dexamethasone, to a donor is done by some clinicians without there being unequivocal evidence of its efficacy. Patients with a profound neutropenia, not expected to recover before long, and an uncontrolled clinically documented infection, such as severe cellulitis or sinusitis, appear to be the primary candidates for treatment with granulocyte transfusions, whereas administration of G-CSF should be favored when a return of the neutrophils is imminent. Significant toxicities in granulocyte-transfusion recipients include transmission of cytomegalovirus, alloimmunisation associated with fever, graft-versus-host reactions if granulocytes are not irradiated, progressive platelet refractoriness, and, possibly, respiratory insufficiency associated with concomitant administration of amphotericin B. While granulocyte transfusion therapy remains an experimental therapeutic intervention, it is clear that appropriate transfusion of thrombocyte- and erythrocyte-suspensions to prevent overt hemorrhage and hypoxemia or heart failure, respectively, offer an important contribution in the battle against infectious complications that occur during the treatment of patients suffering from acute leukaemia. New approaches with agents designed to protect the mucosa, like recombinant human Interleukin 11 and keratinocyte growth factor palifermin, show promising results in terms of reduction of severity of MBI and occurrence of fever and bacteraemia in neutropenic patients.

CESSATION OF ANTIMICROBIAL THERAPY

ANTIBACTERIAL THERAPY

It is widely believed that antibiotic treatment should be continued for a minimum of 7 days or until culture results indicate that the causative organism has been eradicated, infection at all sites has resolved, and the patient is free of major signs and symptoms. Ideally, the neutrophil
Chapter 2

count should be >500 mm$^3$ (0.5 x 10$^9$/l) before treatment is stopped.\textsuperscript{111,146} When no infection has been identified after three days of treatment and the patient has become afebrile for 48 hours in association with a neutrophil count that has exceeded 500 cells/mm$^3$ (0.5 x 10$^9$/l), antibiotic therapy may be stopped.\textsuperscript{22} In addition, if a persistently neutropenic patient has no complaints and displays no clinical, radiological, or laboratory evidence of infection, cessation of antibiotic therapy or a change to oral antimicrobials should be considered after 4 days without symptoms. If antibiotics are discontinued while the patient is still neutropenic, the patients must be monitored closely and intravenous antibiotics restarted immediately on the recurrence of fever or any other evidence of bacterial infection, since infection may have only been suppressed, not eradicated. One should consider continuous administration of antibiotics throughout the neutropenic period in patients who have a profound neutropenia, MBI, or any other identified risk factor. Some experts suggest, in patients in whom hematological recovery cannot be anticipated, a change from the therapeutic regimen to one of the prophylactic schemes after 2 weeks of therapy with intravenous antimicrobials.\textsuperscript{22} When the suspicion of a non-infectious cause of the fever is high, interruption of the antibiotic therapy after ~4 days seems warranted in clinically well patients without any evidence of infection apart from persisting fever. Under these conditions, meticulous monitoring has to be maintained to guarantee the patients timely protection against subsequent infections that are likely to occur.

**ANTIFUNGAL THERAPY**

The decision to start antifungals may appear complex but is not as difficult as the decision to discontinue. If a systemic fungal infection has been identified, the course of antifungal therapy will be determined by the causative agent and the extent of the disease. In patients with pulmonary infiltrates or other suspicious lesions it is essential to see a clinical and, preferably, a radiological response before one may start to ponder cessation of antifungal therapy. However, if no fungal infection is found it is not clear how long antifungal drugs should be administered.\textsuperscript{147} For clinically well patients with prolonged neutropenia, it is suggested that antifungal agents can be stopped after two weeks of treatment, provided that no conspicuous lesions can be found by clinical evaluation or by CT-scanning of the chest and the abdominal organs. In the patient who appears ill or is at high risk, continuation of antifungal therapy throughout the neutropenic episode is recommended.\textsuperscript{22} Conversely, when neutropenic fever subsides, the patient is clinically well, and CT-scan of the abdomen and chest reveals no suspicious lesions, antifungals may be discontinued, particularly when the criterion for commencing antifungal therapy had been simply fever unresponsive to antibiotics. This approach also applies when the presumptive diagnosis becomes questionable during the course of granulocytopenia. When a patient diagnosed with and treated for a proven or probable invasive fungal disease requires further chemotherapy or a bone marrow transplantation, protection against the offending pathogen has to be provided, irrespective of the previous response to antifungal therapy. The risk of relapse of invasive fungal disease is so high that secondary prophylaxis is
warranted, thereby ensuring that a full dose of the most effective antifungal is administered.\textsuperscript{148,149} After introduction of routine CT scanning it became apparent that solitary lesions caused by invasive fungal disease are utterly rare and this observation reduced the enthusiasm for surgical interventions. However, if the number of lesions is limited or a difficult-to-treat pathogen, such as a zygomycosis, has been found, extirpation has to be considered, especially when the lesions are located close to a large vessel.\textsuperscript{150}

**CONCLUSION**

Modern chemotherapy offers hope of a cure to many cancer patients, but it confronts the medical community with continuous new challenges. Infection remains an inevitable side-effect of the myeloablative therapy for acute leukemia and is the principal cause of morbidity and mortality amongst these patients. Optimal care can be delivered only by those who pay scrupulous attention to the patient’s clinical condition and are aware of the evolving therapeutic and diagnostic modalities. It cannot be denied that time remains an important factor in the management of infectious complications but we must try to distinguish more accurately between patients truly in need of immediate therapy and those who are not. Fixed treatment algorithms are only acceptable if they allow individual interpretation and reasoned deviations. Maintaining guidelines that dictate second line treatment of a population of which more than half of patients do not have serious disease is not justifiable in view of the potential adverse events and the economical burden. The demand for an alternative strategy, build on clinical skills, modern and more accurate laboratory tests and imaging techniques, has become apparent and a broad application of this principle may change the scene of antimicrobial treatment in neutropenic patients completely. Overuse of antimicrobial agents, both antibacterial and antifungal, has become all too common in the belief that a broader cover will benefit the patient. Unfortunately, prescription of antimicrobials according to a preset scheme may give a false feeling of confidence with a reduced ambition to make a diagnosis, whereas diagnostic considerations should prevail whenever patients do not respond satisfactorily to an antibacterial regimen. In addition, neutropenia cannot longer be seen as the major compass to steer antimicrobial therapy in a febrile patient because neutropenia is not the one and only factor predisposing for infection. A damaged mucosa and impairment of the T cell-mediated immunity have altered the pattern of causative micro-organisms. This change has not only consequences for the selection of the antimicrobial agents but may also foster development of totally different treatment modalities such as biological response modifiers that might reduce the need for antimicrobial agents. Undoubtedly, unwarranted widespread use of antibiotics has contributed to the development of resistance amongst the micro-organisms. Resistance of previously susceptible pathogens to drugs like penicillins, cephalosporins, glycopeptides, fluoroquinolones and azoles has become all too familiar, but the threat of diminished susceptibility casts an even
longer shadow in the direction of macrolides, carbapenems and quinolones with an extended spectrum of activity. Prophylactic or empiric use of antimicrobial agents is not recommended to make the physician’s life easy but to help the patients most at risk to survive a difficult episode. In this context it is crucial for doctors always to be sceptical about what they are doing whereas they should be very sure about what they are going to do next.

REFERENCES


Managing fever during neutropenia


Managing fever during neutropenia


PART II

INTRODUCTION OF CITRULLINE AS BIOMARKER FOR INTESTINAL MUCOSAL BARRIER INJURY
CHAPTER 3

CITRULLINE AS A BIOMARKER FOR INTESTINAL MUCOSAL BARRIER INJURY IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

Hematopoietic stem cell transplantation (HSCT) is highly effective for treating hematological malignancies, and other disorders. However, preparation for HSCT is often complicated by mucosal barrier injury (MBI). MBI involves the entire digestive tract and plays an important role in post-transplant complications, including inflammatory and infectious complications, malnutrition and the occurrence of graft-versus-host disease.

This review shows that the amino acid citrulline is a reliable biomarker of MBI and can be measured by a simple and relatively inexpensive blood test. Its use enables standardized assessment of MBI which improves the management of MBI-related complications. Classifying the gastrointestinal MBI induced by conditioning regimens by measuring citrulline can help to determine the need for antimicrobial therapy, total parental nutrition, hospital admission and the use of preventive and therapeutic anti-inflammatory therapies for a certain regimen. Furthermore, citrulline can be used for exploring ways to ameliorate MBI.
INTRODUCTION

In recent years it has become increasingly clear that mucosal barrier injury (MBI) following reduced-intensity (RIC) and myeloablative (MA) conditioning to prepare for a hematopoietic stem cell transplant (HSCT) plays an important role in the occurrence of transplant-related complications.\(^1\)\(^-\)\(^3\) Virtually every patient who receives MA therapy for a HSCT develops MBI and the incidence for RIC regimens is approximately 40-50\%.\(^4\)\(^,\)\(^5\) Oral mucositis (OM) is the clinical manifestation of MBI affecting the oral cavity and has been singled out as the worst complication of this type of conditioning.\(^6\) OM is characterized by pain, oedema, erythema, the formation of painful oral lesions as well as dryness and the production of viscous mucus. However, MBI is not restricted to the mouth but affects the entire alimentary tract and is characterized by nausea, vomiting, reduced appetite and watery diarrhoea, any of which can have an important impact on the short-term quality of life. Moreover, in extreme cases, abdominal pain can herald neutropenic enterocolitis. However these signs and symptoms can also be associated with other complications including infection. MBI can only be considered the true aetiology once other causes have been excluded.

MBI may reduce the chance of survival of patients with cancer as a consequence of postponing or reducing the dose of chemotherapy. It can result in transplant-related complications including malabsorption, fever and bacteraemia making necessary the use of antimicrobial agents, nutritional support and analgesics.\(^3\) MBI also has negative consequences for health economics being associated with longer hospital stay, higher hospital charges, and an increase in the 100-day mortality.\(^2\) Nevertheless, MBI has been largely ignored and is regarded as an inevitable complication that patients just have to “grin and bear” as there were no reliable remedies. Besides, MBI eventually resolves spontaneously. Yet, early identification and monitoring of MBI can have important implications. This article sets out to explore how monitoring of the amino acid citrulline can help determine the presence and the course of MBI so as to improve the management of HSCT recipients.

MUCOSAL BARRIER INJURY

1. PATHOGENESIS

MBI of the gastrointestinal (GI) tract induced by RIC and MA regimens to prepare for a HSCT is a complex pathobiological process. The current hypothesis for the development of MBI involves five overlapping phases: initiation phase in which nuclear factor-kappa B (NF-kB) is activated directly by the given conditioning regimen and indirectly from formation of reactive oxygen species, DNA, and non-DNA damage; the up-regulation and message generation phase in which NF-kB leads to the release of cytokines and chemokines (IL-1\(\beta\), IL-6, IL-8,
TNFα, IL-23, interferon-gamma (IFNγ)) by tissue macrophages, dendritic cells, and release of danger-associated molecular patterns (DAMPS) and the secretion of matrix metalloproteinases is stimulated\(^9,10\); an amplification and signalling phase of these cytokines leading to epithelial cell apoptosis and increased mucosal permeability\(^11\); an ulcerative phase with translocation of microbes or pathogen-associated molecular pattern (PAMPS)\(^12-14\) and finally a healing phase in which the damage is restored. Although this model was developed for oral MBI it is most likely also applicable for intestinal MBI.\(^15-16\) Furthermore it is suggested that the deregulation of the microbial homeostasis upon conditioning treatment could influence all the phases of this mucositis model.\(^17\)

The induction of MBI of the GI tract appears central to the pathogenesis that leads to unrestrained inflammation resulting from excessive release of pro-inflammatory cytokines in HSCT recipients. MBI is associated with fever and other inflammatory and infectious complications such as non-cardiogenic pulmonary oedema, idiopathic pneumonia syndrome, engraftment syndrome, graft-versus-host disease (GvHD), sepsis and acute lung injury (Figure 1).\(^18\)

**Figure 1.** Mucosal barrier injury and inflammatory and infectious complications post-HSCT

Chemotherapy and radiotherapy damage the oral and GI mucosa initiating an inflammatory cascade that culminates in mucosal barrier injury (MBI). Activation of NF-κB, release of DAMPS and increased mucosal permeability resulting in the translocation of PAMPS which activate PRR-pathways ultimately result in uncontrolled mucosal inflammation and de-regulated host-microbial interactions. This becomes manifest as SIRS (fever) and contributes to the occurrence of inflammatory and infectious complications including non-cardiogenic pulmonary oedema, idiopathic pneumonia syndrome, engraftment syndrome, acute GI-GvHD, septic shock and acute lung injury.

Abbreviations: NF-κB = Nuclear factor kappa beta, ROS = reactive oxygen species, MMP = matrix metalloproteinases, DAMP = danger-associated molecular pattern, PAMP = pathogen-associated molecular pattern, PRR = pathogen recognition receptor, SIRS = systemic inflammatory response syndrome, GvHD = graft-versus-host disease.
The pathology of MBI involves increased apoptosis located in crypts, followed by hypoplasic villous atrophy and loss of enterocyte height (Figure 2). However endoscopic examination with biopsy is seldom undertaken on a routine basis. Scoring the severity of MBI is therefore based on signs and symptoms, permeability tests or markers.

**Figure 2. Histological findings of mucosal barrier injury**

This figure shows the jejunum of Winstor rats that were injected with methotrexate (MTX, 60 mg/kg intravenously) (right panels) and NaCl 0.9 % intravenously (controls, left panels). Upper pictures show morphology by hematoxylin and eosin (H&E) staining, bottom pictures show goblet cell distribution by alcian blue staining.

MTX-treated rats demonstrate histological signs of mucositis, with profound villous atrophy and blunting with irregular sometimes even vacuolized enterocytes. Furthermore an influx of inflammatory cells into the stroma of villi is seen (see arrows, right upper picture). In the controls (bottom picture on the left) goblet cells (for the production of mucus) were evenly distributed along the crypt-villous axis. Whereas, on the contrary, in MTX-treated rats goblet cells were restricted along the villi or solely present on villous loops (bottom picture on the right) (photograph courtesy of M. Fijlstra).
2. METHODS FOR MEASURING MBI

2.1 Signs and symptoms
Most assessment scales for MBI are focused on OM, since it is relatively easy to recognise. The World Health Organization and the National Cancer Institute- Common Terminology Criteria for Adverse Event (NCI-CTCAE) scales are commonly used. These scoring systems combine objective signs of OM (erythema and ulcer formation) with subjective and functional outcomes (pain and ability to eat). Their reliability is dependent on inter- and intra- observer variation which can be improved by training.

Intestinal MBI presents a quite different challenge as it cannot be seen. The signs and symptoms such as nausea, vomiting, diarrhoea and abdominal cramps affect almost every HSCT recipient at some time during the immediate post-transplant period. Furthermore these symptoms are non-specific and can be caused or influenced by the given conditioning, infections as well as other medications like opioids.

2.2 Permeability tests
Since the principal features of intestinal MBI are loss of epithelial surface and change in permeability, functional tests have been used to gain an impression of the severity and course of the mucosal damage. The permeability of $^{51}$Cr-labeled ethylenediaminetetracetic acid ($^{51}$Cr-EDTA) can be tested and will increase shortly after the start of conditioning. Unfortunately, $^{51}$Cr-EDTA is radioactive and therefore not suitable for routine use. Sugar permeability tests show an increase of absorption two days after starting intensive chemotherapy probably as a result of local inflammation. The peak of gut permeability has been shown to occur 10-14 days after starting intensive chemotherapy. No real difference could be found in the overall pattern of perturbed gut integrity and the absorptive capacity of patients given various myeloablative regimens.

Nevertheless, sugar permeability tests can determine the onset of disruption and dysfunction of the mucosal barrier of the small intestine. Although sugar permeability tests are non-invasive, they are cumbersome and wholly dependent on patient compliance. Furthermore both $^{51}$Cr-EDTA and sugar permeability tests are influenced by extraneous factors such as bowel transit time, gastric emptying and renal function.

2.3 Biomarkers
The most common markers that have been investigated to study MBI are: albumin, intestinal fatty acid binding protein and citrulline.

2.3.1 Albumin
Albumin will decrease over time after starting conditioning and is often used by clinicians as an indicator of MBI. A recent study of Van der Velden et al. showed that albumin levels are
greatly influenced by inflammation confirming it to be a “negative acute phase protein”. After MA treatment, albumin levels followed the course of inflammation induced by GI damage. A decrease in albumin levels was seen also on day 6 after starting conditioning that included anti-thymocyte globulin (ATG) reflecting the cytokine release induced by ATG. These dynamic changes in albumin levels are mostly a result of decreased albumin synthesis in the liver and an extra-vascular shift (capillary leakage) and not caused by malnutrition as albumin has an estimated half-life of 20 days. Consequently albumin is not a suitable marker of intestinal MBI.

2.3.2 Intestinal fatty acid binding protein
The plasma concentration of intestinal fatty acid binding protein (I-FABP) has been shown to be a highly specific and sensitive method for assessing the extent of mucosal injury. I-FABP is a low molecular weight (12-15 kDa) intracellular protein that is only found in the epithelial cells of the intestinal mucosal layer. It is released as soon as the cell membrane integrity is compromised so is not normally found in plasma. FABPs bind and transport fatty acids and protect the cell against their undesirable effects. An increase in plasma I-FABP is seen after chemotherapy and precedes intestinal complaints. This rise is followed by a rapid decline. Tissue expression of I-FABP is relatively low and more sensitive immunoassays need to be developed to fully exploit the potential of this marker and also to establish proper reference values for healthy subjects. Furthermore, a study that looked for the best longitudinal functional markers of the small intestine of piglets after weaning showed marked variability in I-FABP values.

2.3.3 Citrulline
Citrulline is an amino acid that can be measured in the blood. The amino acid is produced predominately by the gut enterocytes and is not a substrate for metabolism but rather functions as a transporter of nitrogen for the kidney where it is converted to arginine (Figure 3). This gives citrulline the very useful property of indicating the loss of functional small bowel epithelial cells i.e. the enterocytes. Hence, citrulline has the potential of being a dependable instrument to measure intestinal MBI.

3. CITRULLINE

3.1 Historical perspective
A century ago, Koga and Odake isolated an amino acid from the juice of watermelon (*Citrullus vulgaris*) and in 1930, Wada defined the chemical formula and structure and named the amino acid citrulline. Two years later, Krebs and Heinseleit reported citrullines’ importance as intermediate in the formation of urea in the liver. However it was almost 70 years before
citrulline found a role in the clinic when Crenn et al. identified it as a marker for enterocyte mass and intestinal failure in humans.\textsuperscript{39}

In 2004, Blijlevens et al. showed citrulline to be a potential marker for intestinal epithelial damage following myeloablative conditioning therapy.\textsuperscript{35} Lutgens et al. showed that citrulline is more sensitive and more specific for measuring small bowel enterocyte loss than sugar permeability tests.\textsuperscript{40}

\textbf{Figure 3.} The interorgan exchanges of citrulline (ARG/GLN-CIT-ARG cycle).

Citrulline is produced in the intestine mainly from glutamine. It is then released in the systemic circulation, i.e. the caval vein. No other site, but the intestine has been identified so far that releases significant amounts of citrulline under physiological conditions. Citrulline is extracted by the kidneys from the blood and used to synthesize arginine.

Abbreviations: ARG= arginine, ORN= ornithine, CIT= citrulline, GLU= glutamate, GLN=glutamine, ARGase= arginase, GLNase= glutaminase, OCT=ornithine carbamoyl transferase, OAT=ornithine aminotransferase, P5Cs= pyrroline-5-carboxylate synthase, ASS= argininosuccinate synthetase, ASL= argininosuccinate lyase.

\textbf{3.2 Biology}

An important distinguishing characteristic of citrulline is the fact that it is not incorporated into proteins. Hence circulating citrulline in the blood depends almost entirely on \textit{de novo} synthesis.\textsuperscript{41}
Daily ingestion of 6 cups of watermelon juice, a rich source of citrulline, for three weeks showed no significant changes in fasting citrulline levels. In normal subjects there is no significant effect of protein intake on plasma citrulline 3 hours after a protein meal. Moreover citrulline is almost exclusively synthesized and released in the circulation by the proximal small bowel (duodenum and jejunum) most likely from the middle and upper parts of the villi. Glutamine is the main precursor of citrulline and accounts for 80% of the production. Arginine and certain other amino acids such as proline also contribute to the intestinal production of citrulline. After it is released into the bloodstream, citrulline passes through the liver virtually unchanged since hepatic metabolism is negligible except under certain pathological conditions such as liver metastasis. Furthermore there is no significant influence on circulating citrulline by systemic or intestinal inflammation. Being a small molecule, citrulline is easily filtered by the renal glomerulus and subsequently reabsorbed and metabolized in the proximal tubule. Approximately 83% of the citrulline released by the gut is metabolized by cells of the proximal tubules of the nephron and converted into arginine, which is then converted to nitrogen (Figure 3). Consequently, the concentration of circulating citrulline depends only on the production and release of citrulline by the enterocytes mass and on renal excretion. Individuals with normal intestinal mucosal function and normal renal function given a Western diet have citrulline levels between 30 and 50 μmol/L with a median of 40 μmol/L, as determined by ion-exchange chromatography. An increase in citrulline levels is seen in kidney failure and severe renal failure is characterized by hypercitrullinaemia.

3.3 Function
Citrulline measured in serum or plasma can therefore act as a potential marker for the mass of viable or metabolically active enterocytes, with lower values reflecting an impaired enterocyte function. Theoretically, increased utilization could lead to lower values though this has never been described. Importantly, the concentration of circulating citrulline does not indicate an specific aetiology but is solely a marker of intestinal damage. Cittu et al. showed that citrulline levels correlate with the severity and extent of villous atrophy disease by comparing plasma citrulline concentrations with the anatomical enterocyte mass estimated from small bowel length and villous height of patients with coeliac disease and other causes of small bowel villous atrophy. A citrulline level < 10 μmol/L is associated with total villous atrophy and defines hypocitrullinaemia. Citrulline levels of 10-20 μmol/L are predictive only of proximal villous atrophy with a citrulline level > 20 μmol/L indicating partial villous atrophy. In patients with small bowel syndrome, plasma citrulline is correlated with the length of the residual small bowel. Citrulline concentrations are consistent with small bowel absorption capacity.
### Table 1. Function of citrulline as marker of enterocyte mass in several patient populations

<table>
<thead>
<tr>
<th>CITRULLINE THRESHOLD (MICROMOL/L)</th>
<th>PATIENT POPULATION</th>
<th>SIGNIFICATION OF</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>Pts with villous atrophy disease</td>
<td>Total villous atrophy&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HSCT recipients, first 30 days</td>
<td>Increased risk of bacteraemia&lt;sup&gt;61&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HSCT recipients, &gt; 30+ days post-allogeneic transplantation</td>
<td>Increased risk of GI aGVHD&lt;sup&gt;75,76&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pts with HIV disease</td>
<td>Indication for parenteral nutrition&lt;sup&gt;47&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt; 11</td>
<td>Pts with short bowel syndrome</td>
<td>Indication for parenteral nutrition&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td>&gt; 11</td>
<td>Pts with HIV disease</td>
<td>Weaning off parenteral support / Indication for enteral support&lt;sup&gt;47, 67&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pts with short bowel syndrome</td>
<td></td>
</tr>
<tr>
<td>&lt; 13</td>
<td>Recipients of intestinal transplant, three months ago</td>
<td>Graft rejection or severe infection&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥ 13</td>
<td>Recipients of intestinal transplant, three months ago</td>
<td>No moderate or severe rejection&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
<tr>
<td>10-20</td>
<td>Pts with villous atrophy disease</td>
<td>Proximal only total or subtotal villous atrophy&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>Pts with short bowel syndrome, past the 2-year adaptive period</td>
<td>Permanent chronic intestinal failure&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>Pts with villous atrophy disease</td>
<td>Partial villous atrophy&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pts with short bowel syndrome, past the 2-year adaptive period</td>
<td>Transient chronic intestinal failure&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: Pts= patients, HSCT= hematopoietic stem cell transplantation, GI GvHD= gastro-intestinal graft versus host disease, HIV= human immunodeficiency virus.

Furthermore during follow-up after small bowel transplantation, citrulline levels were shown to be a potent indicator of acute cellular rejection.<sup>52,53</sup> Regular monitoring of serum samples during the pre-and postoperative period after intestinal allograft has now become common practice (Table 1).

#### 3.4 Measurement

Citrulline can be determined in the blood by methods based on high pressure liquid chromatography either using an ion exchange column or a reversed phase column.<sup>54,55</sup> These methods have been compared in the past and the results of these studies have been established the equivalence of these methods.<sup>56</sup> Furthermore, there is no significant difference in results or interpretation between analytical methods using serum or plasma for determination of citrulline.<sup>43</sup> A fast and rapid method for measuring plasma citrulline was recently reported.
using ultra-performance liquid chromatography tandem mass spectrometry.\textsuperscript{57} Citrulline concentrations can be determined in as little as 10 microL of plasma or serum within 30 minutes of receipt of the sample and at least 12 assays per hour can be determined. Postal transport to the laboratory is also feasible as citrulline is stable in plasma and serum for at least 2 days at room temperature. A single measurement of citrulline is sufficient and timing is not important as levels do not change much during the course of the day for HSCT recipients as they tend not to eat and are sustained by parenteral nutrition. Each assay costs as little as 20 euro per sample.

There are two citrulline-based assessment scores. One is based on the level of citrulline using severity thresholds, and the second on the area under the reciprocal ($10/$\text{citrulline (μmol/l)}) curve. Both scores are able to discriminate between damage induced by different conditioning regimens. The score based on the area under the reciprocal curve of citrulline requires only a few measurements to estimate the score, but is probably only appropriate for research purposes. For clinical purposes, a scoring system based on absolute citrulline concentrations is more practical for determining intestinal MBI.\textsuperscript{58}

4. CITRULLINE AS BIOMARKER FOR MBI FOLLOWING CONDITIONING FOR HSCT

Citrulline levels decrease in proportion with the damage to the mucosa following conditioning treatment. Monitoring citrulline helps to understand the process of MBI in relation to post-transplant complications so that certain complications can be anticipated and, if necessary, appropriate changes in management can be arranged.

4.1 Defining the course of MBI following conditioning

A rapid decline in circulating citrulline is seen soon after the start of conditioning therapy, which first reaches a nadir, after which there is a gradual increase back to normal levels.\textsuperscript{33} Citrulline does not appear to be influenced by inflammation in adults or children.\textsuperscript{28,59,60} By using citrulline, it is possible to discriminate between the severity and duration of intestinal MBI induced by different conditioning regimens.\textsuperscript{3} MA regimens are associated with severe and prolonged intestinal damage shown by a rapid decline in citrulline to < 10 μmol/L a mean of 10 days after starting chemotherapy with hypocitrullinaemia usually lasting for a week or so. By contrast, there is an early but short decline in citrulline levels following non-myeloablative (NMA) conditioning though few patients develop hypocitrullinaemia.\textsuperscript{3,61}

5. ROLE OF MONITORING CITRULLINE TO GUIDE THE APPROACH REGARDING MBI-RELATED POST-TRANSPLANT COMPLICATIONS

5.1 Risk prediction for fever and infections

MBI has been shown to be the main determinant of fever and inflammation in HSCT recipients.\textsuperscript{3} This means that some HSCT recipients develop fever simply as manifestation of MBI-related
inflammation and not infection. The term “febrile mucositis” has been proposed to distinguish this from “febrile neutropenia”. Indeed, bacteraemia appears to develop as a result of severe damage to the intestine, i.e. during hypocitrullinaemia. MBI probably allows bacteria to easily translocate from the gut lumen into the blood due to the villous atrophy that occurs. The bacteraemia that occurs can, in some cases, result in fulminant sepsis. Indeed, studies of Costa et al. and Ruescher et al. confirmed that bacteraemia in HSCT recipients typically originates from the gut. MBI therefore represents a breach in the first line of defence but, since it occurs simultaneous with neutropenia, the second line of defence consisting of phagocytic cells is no longer able to prevent translocation or dissemination.

Citrulline levels are able to distinguish between the MBI induced by different conditioning regimens. This allows the period of increased risk for inflammatory and infectious problems to be predicted as it coincides with the occurrence of severe intestinal damage. For MA conditioning, the period of high risk for infections can be expected to begin around 10 days after the start of the conditioning regimen which would be a signal to start taking extra measures such as more intensive monitoring of vital signs including body temperature to help recognise complications early (Figure 4, Table 2). Currently, such intensive controls are usually started as soon as neutropenia starts or even earlier. With a relative short period of hypocitrullinaemia the burden on nursing personnel would be less releasing them for other tasks. Furthermore, better planning of the HSCT could ensure that the period of greatest risk does not occur during the weekend. Hence, simply knowing that infectious problems are most likely to occur during the week makes managing HSCT recipient outside the hospital feasible and safe provided it is accompanied by regular outpatient visits. HSCT recipients will also be less exposed to other risk associated with weekends such as limited access to supportive facilities.

The expected risk of developing inflammatory and infectious problems for patients receiving a NMA regimen is much lower than in those treated with MA treatment and few patients do develop bacteraemia. Hence there is even less reason to keep them in hospital during the immediate post-transplant phase.

Since fever could be the result either of bacteraemia or local infection or MBI inflammation empirical treatment with parenteral antimicrobial therapy should still be instituted though it cannot be predicted who will benefit and who will not. However, if fever can be shown to be related solely to MBI inflammation rather than infection, currently considered fever of uncertain origin, simply switching or adding antimicrobial agents would be futile and should be discouraged. Instead, every attempt should be made to diagnose or exclude microbiologically and clinically defined infections. Further therapy could then be directed by the findings whether they confirm or exclude an infectious aetiology.

Incorporating citrulline into the standard of care for managing fever and infections could reduce the work-load for nursing personnel, hospital admissions, the length of stay, and overuse of antimicrobial treatment thereby lowering the risk of emerging antimicrobial resistance.
Introduction of citrulline as biomarker

Figure 4. Course of citrulline reflecting intestinal MBI & its relationship with neutropenia, inflammation, bacteraemia and nutritional support in a myeloablative regimen

Citrulline concentrations rapidly decline after the given MA regimen, reflecting intestinal MBI. A nadir is reached, which is followed by a gradually increase back to normal levels. There is a striking pattern of inflammation (shown by the course of CRP) that in time correlates with the occurrence of intestinal MBI. Fever (>80%) and bacteraemia (30-50%) coincide with the presence of severe intestinal MBI, designated by hypocitrullinaemia (citrulline values below 10 μmol/L). Neutropenia (ANC ≤ 0.5 x 10⁹/L) is reached prior to hypocitrullinaemia, and the period of neutropenia is also longer than the period of hypocitrullinaemia. However no major impact of neutropenia has been seen on occurrence of fever and bacteraemia in former studies. In patients who are not eating, the choice of nutritional support can be determined by the course of citrulline. Parenteral nutrition should especially be given to patients that receive a myeloablative regimen that induce a long duration of hypocitrullinaemia. Citrulline above a certain level will allow weaning off parenteral nutrition.

Abbreviations: EN= enteral nutrition, PN= parenteral nutrition.

Table 2. Clinical advice based on citrulline thresholds in HSCT recipients during the first 30 days after transplant

<table>
<thead>
<tr>
<th>CITRULLINE THRESHOLD (MICROMOL/L)</th>
<th>CLINICAL RECOMMENDATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>Indication for:</td>
</tr>
<tr>
<td></td>
<td>• (Prophylactic) antimicrobials</td>
</tr>
<tr>
<td></td>
<td>• Switch from oral to i.v. administration of several drugs, including quinolones</td>
</tr>
<tr>
<td></td>
<td>• Parenteral nutritional support</td>
</tr>
<tr>
<td></td>
<td>• Increased monitoring of vital controls</td>
</tr>
<tr>
<td></td>
<td>• Administration of drugs that ameliorate MBI, if available</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>Possible indication for:</td>
</tr>
<tr>
<td></td>
<td>• Weaning off parenteral nutrition</td>
</tr>
<tr>
<td></td>
<td>• Starting enteral support</td>
</tr>
<tr>
<td></td>
<td>• Outpatient treatment</td>
</tr>
</tbody>
</table>
5.2 Guidance of nutritional intervention

Patients that receive a HSCT often need nutritional support. Whereas low dose enteral nutrition is better for the trophic effects, more calories can be given by total parenteral nutrition (TPN). MBI is likely to be the main determinant for choosing not only nutritional support (because the complaints that are caused induce anorexia) but also how it is given. TPN is still the only option when there is severe malfunction of the gut, GI-fistulae or prolonged ileus but patients will benefit more from enteral support once intestinal reconstitution occurs. Measuring citrulline could be valuable to determine the best approach regarding nutritional support as it has shown to be consistent with small bowel absorption capacity. It is also conceivable that patients receiving MA regimens might benefit most from TPN as the chemotherapy they are given induces a protracted period of hypocitrullinaemia. Patients can be weaned off parenteral nutrition once citrulline attains a certain level. For instance, for patients with HIV none with a plasma citrulline concentration < 10 μmol/L could be weaned of parenteral nutrition. By contrast, a study among children with short bowel syndrome weaning off parenteral nutrition was possible above a level of 11 μmol/L (Table 1).

Moreover, TPN is probably only necessary for a limited time after MA conditioning (Figure 4, Table 2). Patients given NMA regimens probably benefit more from enteral nutrition. Studies are eagerly awaited in HSCT recipients to investigate this more thoroughly. If citrulline levels are able to guide the best approach for nutritional support this will most likely lead to a reduction of TPN use as well as thrombosis and catheter related infections.

5.3 Guidance on the route of administration of drugs, including antimicrobials

Intestinal damage due to MBI can affect the absorption, resorption or excretion of medication although this is generally overlooked. Intestinal metabolic enzymes, such as cytochrome P 450 (Cyp3A), are abundant in the intestinal epithelium present. Damage to the GI mucosa could therefore influence the bioavailability of certain medications positive or negatively depending on the uptake mechanisms and intracellular distribution. Citrulline levels could be useful in determining the best route for administering medication during the different stages of MBI in order to obtain the optimal effect. For example, the small intestine is the site of absorption of the quinolones which are given orally as antimicrobial prophylaxis in HSCT recipients. In healthy subjects the oral bioavailability of ciprofloxacin and ofloxacin is, respectively, 55-85% and almost 100%. However malabsorption will occur when there is mucosal damage leading to a significant lower exposure (Table 2). Hence, administration of quinolones should change from the oral to the intravenous route once a certain threshold of citrulline levels is attained in order to maintain therapeutic drug levels, which deserves further investigation.

5.4 Role of citrulline in graft-versus-host disease of the gastrointestinal tract

GvHD is frequently seen after allogeneic HSCT suggesting another role for biomarkers of gut damage. Acute GvHD is a leading cause of non-relapse mortality and morbidity following
allogeneic HSCT and primarily affects the skin, liver and GI tract. Acute GvHD results from an allo-immune response directed at host antigens and is accompanied by the activation of alloreactive T-lymphocytes that attack the host tissues. MBI following conditioning plays an important role in the initiation phase of acute GvHD. The incidence of acute GvHD ranges from 35-80%, and is highly dependent on the intensity of conditioning, the donor type, HLA match, and graft characteristics. GvHD affects approximately 30-50% of cases with GvHD but is often clinically indistinguishable from other causes of GI dysfunction such as conditioning regimen-related toxicity, infection and drug side effects, as they all can present with diarrhoea. The diagnosis of GvHD relies on the production of profuse diarrhoea together with specific histology of gut tissue obtained by biopsy in the absence of infections such as *Clostridium difficile* and cytomegalovirus. The severity of acute GvHD is graded clinically from I-IV using a standardized system that evaluates three principal target organs, with increased mortality rates in severe acute GvHD (grade III-IV). GvHD is graded on the volume of diarrhoea, but scoring diarrhoea is difficult, cumbersome and unpleasant. Moreover, stool volume can be affected by many factors including oral intake, the use of opioids and concomitant infection. Consequently, several studies have focused on the use of biomarkers to detect, grade and predict the outcome of acute GvHD.

Measuring citrulline before engraftment might predict those who are at risk for GI-GvHD since MBI following conditioning is a predisposing factor. Indeed, Van der Velden et al. showed that profound hypocitrullinaemia following allogeneic HSCT is correlated with the occurrence of acute GI GvHD. In this study none of the patients treated with a NMA conditioning regimen developed severe acute GvHD unlike 6-10% following MA therapy who developed the complication early suggesting citrulline could be used to predict it. Low citrulline levels have also been observed in paediatric patients suffering from GI-GvHD. Vokurka et al. reported significantly low citrulline levels among a group of patients considered as suffering from GI-GvHD 43-142 days post-transplant. Merlin et al. evaluated citrulline as a marker for acute GI-GvHD in 31 children undergoing an allogeneic HSCT of whom 19 had been diagnosed with GvHD, seven with GI symptoms. Hypocitrullinaemia one month or longer after the start of conditioning therapy correlated strongly with the occurrence of acute GI-GvHD (Table 1). Furthermore, citrulline levels increased in 3 of the 7 cases with acute GI-GvHD after initiating GvHD therapy but before the clinical symptoms improved. A study of Gosselin et al. showed significantly lower citrulline levels in subjects with acute GI-GvHD. It is also possible that citrulline may be a preclinical marker of GvHD since the nadir in citrulline concentration (median day 10) preceded the diagnosis of acute GvHD (median day 19). This suggests that citrulline can be used as an early marker for the response of GvHD. Furthermore, no increase in citrulline levels after the start of GvHD treatment could be a sign of refractory GvHD. However, further studies are needed to explore this and assess the value of citrulline monitoring compared to other markers including albumin and Regenerating Islet-derived Protein 3-alpha (REG-3α).
6. ROLE OF CITRULLINE IN GASTROINTESTINAL INFECTIONS

HSCT recipients can develop GI infections caused by bacteria (e.g. Clostridium difficile), viruses and less frequently, protozoa and parasites. Citrulline levels may also prove a general indicator of intestinal mucosal dysfunction when these GI infections induce severe damage to the mucosa. Intestinal transplant rejection was established by measuring citrulline and viral enteritis has been shown to affect citrulline levels too. This should provide an impetus for study among HSCT recipients with mucosal damage due to other transplant-related complications.

7. ROLE OF CITRULLINE IN THE INVESTIGATION OF STRATEGIES TO AMELIORATE MUCOSAL BARRIER INJURY

Unfortunately there are few remedies available to ameliorate MBI. Palifermin was shown to have some effect on OM in patients treated with a TBI containing regimen, but the effect on intestinal MBI was not clear. The use of growth factors to stimulate intestinal stem cells, e.g. R-spondin-1, IL-22 and glucagon-like peptide that are now being considered as potential tests for acute GI-GvHD could be of interest for managing MBI, as could modulations in the gut microbiota. Guidelines for managing intestinal MBI already suggest that treatment with probiotics containing Lactobacillus species may have a protective effect against chemotherapy- and radiotherapy induced diarrhoea in patients with pelvic malignancies. Another approach might be to exploit pathways that enable tissue repair and resolution of the inflammatory process, by using anti-inflammatory cytokines (such as interleukin 10), or cytokines inhibitors (such as IL-1Ra), or scavengers that neutralize the effects of PAMPs and DAMPs (like anti-HMGB-1), or by correcting deficiencies in antimicrobial peptides (e.g. lactoferrin and defensins). A citrulline-based score could be used as an objective biomarker to evaluate the effect of these potentially useful strategies to prevent or treat MBI.

FUTURE DIRECTIONS

Citrulline is a valuable biomarker of intestinal MBI in HSCT recipients and allows for a standardized assessment of MBI. This can support the management of MBI-related complications. Citrulline levels can also be used to guide clinical decisions since the severity and duration of MBI plays an important role in the need for antimicrobial therapy and nutritional support in HSCT recipients as well as determining the optimal route for administering medication. Citrulline may also have a role in measuring the intestinal damage associated with GI-GvHD and in monitoring the response to treatment thereby allowing timely adjustments in the case of failure. Furthermore, citrulline can be used to explore remedies to ameliorate MBI. Future studies are needed to determine the specific thresholds of citrulline levels at which certain actions should be taken or the course of actions changed. GI infections should be investigated in relations to citrulline levels. Clearly, formal studies are necessary to evaluate...
whether actions based on the severity of MBI really do lead to the expected improvements in the management of HSCT recipients in terms of reduced morbidity and mortality, better quality of life and lower health care costs.

REFERENCES


Introduction of citrulline as biomarker


89. Carvalho FA, Aitken JD, Gewirtz AT, Vijay-Kumar M. TLRS activation induces secretory interleukin-1 receptor antagonist (sIL-1Ra) and reduces inflammamsome-associated tissue damage. Mucosal Immunol 2011; 4: 102-111.

CHAPTER 4

CITRULLINE-BASED ASSESSMENT SCORE: FIRST CHOICE FOR MEASURING AND MONITORING INTESTINAL FAILURE AFTER HIGH-DOSE CHEMOTHERAPY

ABSTRACT

INTRODUCTION
Currently, objective tests are lacking that enable the extent and duration of intestinal mucosal injury (MBI) induced by myeloablative (MA) chemotherapy to be determined. To address this problem we explored a citrulline-based assessment score as this amino acid is a simple quantitative marker of intestinal failure.

PATIENTS AND METHODS
From March 2004 to June 2007 citrulline concentrations were determined at baseline and at least once weekly after start of MA chemotherapy until 30 days thereafter among 94 allogeneic or autologous hematopoietic stem cell transplant recipients. The patients were divided into three groups according to the regimen they received: (1) BEAM/HDM, (2) Cyclo-TBI+-ATG and (3) idarubicin-containing regimens. Intestinal MBI was described either by level of citrulline on each day, based on different thresholds of citrulline indicating the severity of villous atrophy, and by AUC using reciprocal value of 10/citrulline.

RESULTS
Regimens that incorporated idarubicin induced the most severe intestinal toxicity. Scores based on level of citrulline, using severity thresholds and on the area under the reciprocal curve are able to discriminate between the damage induced by different high-dose chemotherapy regimens.

CONCLUSION
A citrulline-based assessment score appears objective, validated, reproducible, reliable, specific and sensitive making it a suitable first choice for measuring and monitoring intestinal MBI.
INTRODUCTION

Mucositis, also called mucosal barrier injury (MBI) is a common adverse effect of myeloablative chemotherapy or radiotherapy used to prepare patients for a hematopoietic stem-cell transplant (HSCT).\(^1\) Patients describe oral MBI as the most debilitating complaint though the entire alimentary tract is affected. Clinical consequences of MBI include dehydration, malnutrition, potentially life threatening infections and possibly even increased mortality.\(^2\) MBI can have a direct impact on morbidity-prolonged hospital stay, increased antibiotic usage and the need for parental nutrition, and is a serious economic burden.\(^3\)

Consequently, early detection, assessment and monitoring of mucosal damage is necessary for effective management. Therefore ideally we should have a scoring system that is objective, validated, reproducible, reliable (without inter- and intra-observer variation), sensitive and precise, and requires minimal training as set out by the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology (MASCC/ISOO).\(^4\)

Most assessment scales for mucosal damage are focused on oral MBI, since it is easy to recognize. Both the WHO scale and the NCI/CTCAE (National cancer institute/ common terminology criteria for adverse event) version 3.0 scale are commonly used.\(^5,6\) These combine objective signs of mucositis (erythaema and ulcer formation) with subjective and functional outcomes (pain and the ability to eat). Their reliability is dependent on inter- and intra-observer variation, which can be improved by training.\(^7\)

Intestinal MBI presents a different challenge as it cannot be seen or readily detected. Endoscopy with or without biopsy is often precluded because of the high likelihood for bleeding complications as MBI develops contemporaneously with bone marrow aplasia, so patients are also profoundly thrombocytopenic.\(^8\) Certain non-invasive tests such as the sugar permeability tests can detect alterations in permeability due to loss of the epithelial surface, but are cumbersome, wholly dependent on patient compliance and cannot distinguish between mucosal damage induced by different myeloablative regimens.\(^9\) The CNI/CTCAE version 3.0 scale is considered the best scale for intestinal MBI\(^10\) and is based on signs and symptoms related to gastrointestinal (GI) changes, including nausea, vomiting and diarrhea.\(^5\) However this scale suffers from several drawbacks that include a lack of reliability and validation, as the signs and symptoms are influenced by the use of antiemetics and opioids for analgesia, which induce constipation. Furthermore, the score is neither specific nor objective.

Consequently, the lack of a diagnostic tool to measure intestinal MBI impedes good clinical management and hampers the development of clinical studies. We and others have explored using the amino acid citrulline as it can be determined in blood and has been shown to be a reliable and objective biochemical marker of small bowel enterocyte mass.\(^11-16\) Injury to the small intestine after high-dose chemotherapy is characterised by crypt apoptosis, hypoplastic villous atrophy and loss of enterocytes, and can be measured by the decline in circulating citrulline with low concentrations corresponding with severe intestinal damage.\(^12,17\) In patients
with small bowel disease and recipients of an intestinal transplant citrulline provides an indication of global function and a useful nutritional prognosis.\textsuperscript{18} We therefore undertook an observational audit to explore an assessment scale for intestinal mucosal barrier damage based on the levels of circulating citrulline that had been measured in patients who had received a T-cell depleted allogeneic HSCT or an autologous HSCT to treat a hematological malignancy.

**PATIENTS AND METHODS**

**PATIENTS**

Between March 2004 and June 2007 a cohort of 94 patients participated. 51 patients had received a T-cell depleted allogeneic HSCT (including 20 recipients of a matched unrelated donor (MUD) transplant) and 43 patients who had received an autologous HSCT.

The myeloablative (MA) regimens consisted of high dose melphalan (HDM), carmustine, etoposide, cytarabine and melphalan (BEAM), cyclophosphamide, antithymocyte globulin and total body irradiation (Cyclo-ATG-TBI), cyclophosphamide and total body irradiation (Cyclo-TBI), idarubicin, cyclophosphamide and total body irradiation (Ida-Cyclo-TBI) and idarubicin, busulfan and cyclophosphamide (Ida-Bu-Cyclo). On admission all transplant recipients had a central venous catheter inserted. Parenteral nutrition was started during conditioning. Cyclosporine was given to allogeneic HSCT recipients for prophylaxis against graft-versus-host disease (GvHD). Patients were given ondansetron for antiemesis. Hematopoietic growth factors were not used. Anti-microbial prophylaxis and therapy were given according to a standard protocol and consisted of valaciclovir and ciprofloxacin. Agents that might ameliorate MBI were not given. Renal function was determined to allow for dosage adjustment of melphalan when the creatinine clearance was lower than 30 mL/min and to identify high concentrations of citrulline which increase when creatinine clearance falls below 50 mL/min.\textsuperscript{19} The glomerular filtration rate (GFR) was estimated thereafter from the serum creatinine using the formula of Cockcroft and Gault.\textsuperscript{20}

**CITRULLINE MEASUREMENTS**

Since the MA therapy determines the severity of MBI\textsuperscript{21}, plasma was obtained at baseline i.e. before the start of the conditioning regimen and at least once per week after the start of the regimen till 30 days. Plasma was stored at –80 °C until required. Citrulline concentrations (μmol/L) were measured by a standard procedure for determining amino acids using high-performance liquid chromatography (Shimadzu©).\textsuperscript{22}

**DATA-ANALYSIS**

The patients were divided into three groups, according to the MA regimen: (group 1) BEAM or HDM, (group 2) Cyclo-TBI + ATG and (group 3) all regimens containing idarubicin.
Citrulline was described in two ways:
a. by the level of citrulline on each day (mean ± SD), based on different thresholds of citrulline. We applied thresholds that indicate severity of villous atrophy documented in patients with coeliac disease, i.e. citrulline level <10 μmol/L is considered predictive of total villous atrophy, citrulline level 10-20 μmol/L is predictive of proximal only total or subtotal villous atrophy and >20 μmol/L indicating only partial villous atrophy. The nadir of each regimen was determined and the duration and frequency of a citrulline below 10 μmol/L was calculated.
b. by the AUC using rescaled reciprocal of the citrulline value (10/citrulline) analogous to the analysis reported by Wardley et al, who showed that for oral MBI MA regimens could be distinguished according to the area under the oral MBI curve.

We modelled the 10/citrulline profile over the first 30 days (or until discharge if this occurred earlier) after the start of conditioning for each patient using linear mixed models treating the ‘patient’ as a random factor, ‘conditioning regimen’ as a fixed factor in combination with a six degrees polynomial function of time. Using this modelling approach we could adequately deal with missing values resulting from only 3 or 4 citrulline measurements per week per patient. One-way Analysis of Variance or the Kruskall-Wallis test was used to compare the three groups with respect to continuous variables. The severity of intestinal MBI was measured in each regimen by depth of citrulline curve (nadir), the duration of citrulline below 10 μmol/L, and the area under the 10/citrulline curve. In order to develop an assessment scale we sought to discriminate between the different regimens by using the two different approaches. A P-value of <0.05 was considered to indicate significance. SAS version 8.2 software (SAS, Inc. Cary, NC, USA) was used for statistical analysis.

RESULTS

The mean age of the 94 patients was 49 (range 17-65) years. Group 1 consisted of 40 patients who received an autologous HSCT. 29 patients were treated for multiple myeloma with HDM. 11 patients were treated for non- Hodgkin’s lymphoma with BEAM. Group 2 consisted of 29 patients who were treated with Cyclo-TBI + ATG. 8 received a sibling donor allogeneic HSCT and were treated with Cyclo-TBI. 21 patients received a MUD transplant, and were treated with Cyclo-ATG-TBI. Group 3 contained 25 patients treated for several hematological diseases with Ida-Bu-Cyclo or Ida-Cyclo-TBI preceding a HSCT. Three patients in group 3 received an autologous HSCT and 22 patients an allogeneic HSCT. The demographic data of the HSCT recipients divided into the three groups, according to the MA regimen are summarized in Table 1.

There were no treatment-related deaths and every patient had a GFR above 50 ml/min, hence there was no need to adjust the dose of melphalan or to correct citrulline concentrations for renal dysfunction.
## Table 1. Demographic data of HSCT recipients treated with myeloablative regimens

<table>
<thead>
<tr>
<th>Group</th>
<th>Regimen Details</th>
<th>Doses</th>
<th>Freq</th>
<th>Days</th>
<th>Type of HSCT (Day)</th>
<th>M/F</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (N = 40)</td>
<td>(\text{HDM}) Melphalan</td>
<td>100 mg/m²</td>
<td>od</td>
<td>1, 2</td>
<td>Autologous, Day 4</td>
<td>17/12</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>(\text{BEAM}) Carmustine Etoposide Cytarabine Melphalan</td>
<td>300 mg/m²</td>
<td>od</td>
<td>1</td>
<td>Autologous, Day 7</td>
<td>7/4</td>
<td>NHL</td>
</tr>
<tr>
<td>2 (N = 29)</td>
<td>(\text{Cyclo-TBI}) Cyclophosphamide TBI</td>
<td>60 mg/kg</td>
<td>od</td>
<td>1, 2</td>
<td>Allogeneic, Day 7</td>
<td>6/2</td>
<td>NHL/CLL (7) MDS/AML (1)</td>
</tr>
<tr>
<td></td>
<td>(\text{Cyclo-ATG-TBI}) Cyclophosphamide ATG TBI</td>
<td>60 mg/kg 2 mg/kg</td>
<td>od</td>
<td>1, 2</td>
<td>MUD, Day 9</td>
<td>16/5</td>
<td>NHL/CLL (6) CML (3) MDS/AML (9) ALL (2) Myelofibrosis (1)</td>
</tr>
<tr>
<td>3 (N = 25)</td>
<td>(\text{Ida-Bu-Cyclo}) Idarubicin Busulfan Cyclophosphamide</td>
<td>42 mg/m² 60 mg/kg</td>
<td>In 48h</td>
<td>1</td>
<td>Allogeneic (12) Autologous (2) Day 13</td>
<td>12/2</td>
<td>CML (1) MDS/AML (10) ALL (3)</td>
</tr>
<tr>
<td></td>
<td>(\text{Ida-Cyclo-TBI}) Idarubicin Cyclophosphamide TBI</td>
<td>42 mg/m² 60 mg/kg</td>
<td>In 48h</td>
<td>1</td>
<td>Allogeneic (10) Autologous (1) Day 13</td>
<td>10/1</td>
<td>NHL/CLL (4) MDS/AML (5) ALL (1) M.Waldenstrom (1)</td>
</tr>
</tbody>
</table>

Abbreviations: ATG: anti-thymocyte globulin, Gy = gray, TBI = total body irradiation, od = once daily; bd = two times daily; MM = multiple myeloma, NHL = Non-Hodgkin’s lymphoma, CLL = Chronic lymphocytic leukemia, MDS = myelodysplastic syndrome, AML = acute myeloid leukaemia, ALL = acute lymphocytic leukaemia.

The course of observed citrulline means in the three groups is shown in Figure 1. Mean citrulline at start of the conditioning regimen for all patients was: 22.1 μmol/L ± 8.1 (mean ± SD). No significant differences were found at baseline between the three groups (P= 0.276). A significant decrease was seen in all groups immediately after the start of MA therapy, with citrulline reaching 10 μmol/L around day 9 (Group 1: 9.6 ± 3.2, Group 2: 8.4 ± 3.3, group 3: 9.4 ± 4.3). A nadir was reached respectively on day 14.4 ± 2.6 for group 1, day 15.3 ± 4.7 for group 2 (disregarding the outlying mean value at day 10, based on a single observation) and day 16.5 ± 2.9 for group 3 (with a significant difference between groups 1 and 3: P=0.008 Mann-Whitney-U). All patients in group 3 had citrulline values below 10 μmol/L (reflecting total villous atrophy) for at least one day, compared to 88% (35 out of 40 patients) in group 1 and 84% (21 out of 25 patients) in group 2. During the first 30 days after the start of the conditioning, patients in
group 3 experienced 21 ± 5 days of citrulline below 10 μmol/L in comparison to 16 ± 7 days for group 1 and 17 ± 7 days for group 2 (Kruskal-Wallis; P=0.005).

The conditioning regimens incurring the most severe intestinal MBI were those that incorporated idarubicin based on the course of citrulline. Group 3 had on average lower citrulline levels starting from day 13 compared to groups 1 and 2, and based on the AUC. The mean area under the modelled 10/citrulline-curve of group 3 was 44.7 (day/μmol/L) compared to 32.8 (day/μmol/L) for group 1 and 32.6 (day/μmol/L) for group 2 (P<0.0001, one-way ANOVA; Figure 2).
DISCUSSION

This observational audit shows that the course of citrulline is able to discriminate between different regimens. This makes a score based on citrulline more specific and sensitive than either the NCI-CTCAE assessment score or sugar permeability tests.\(^5,9\) Citrulline as a marker for regimen-induced intestinal damage is highly reproducible, showing the same course of citrulline for each single patient treated in a certain regimen. Furthermore, citrulline is a quantitative and objective value, lacking inter- and intra-observer variation.\(^12,23\)

Low citrulline concentrations represent intestinal failure independent of the underlying cause and also correlate with the clinical condition of different diseases including small bowel disease, villous atrophy diseases, immunodeficiency virus enteropathy or severe intestinal infectious disease.\(^18\)

In adult patients with short bowel syndrome a citrulline threshold of 20 μmol/L permits the classification into either transient (N=20) or permanent (N=37) chronic intestinal failure, with
Citrulline based assessment score

92% sensitivity, 90% specificity, 95% positive and 86% negative predictive value respectively.\textsuperscript{15} Almost the same is true for children (citrulline cut-off: 19 μmol/L).\textsuperscript{24} Circulating citrulline concentrations can also help evaluate the graft rejection three months after intestinal transplant, the sensitivity for the detection moderate or severe acute rejection was high (sensitivity of 96%; specificity 68,6%, negative predictive value > 99%) when a < 13 μmol/L was adopted as a cut-off.\textsuperscript{16} Thus an assessment score based on citrulline is objective, validated at least by analogy, reproducible, reliable, specific and sensitive and meets the criteria proposed by MASCC and ISOO\textsuperscript{4} better than any other scoring systems to measure intestinal MBI.

Moreover, an assessment score can be based either on the level of citrulline or on the area under the curve. Both are able to discriminate between different regimens which is important for research and practical purposes.

The area under the \(10/\text{citrulline}\) is probably only appropriate for research purposes since the MA regimen is the main determinant of the course of intestinal MBI\textsuperscript{21}, and only a few measurements will be necessary to estimate the AUC. This version resembles the oral mucositis assessment scale (OMAS) designed as a research tool for determining and following the progress of oral MBI.\textsuperscript{25} The OMAS measures a consensus of indicators of oral MBI severity and both the mean mucositis score and the extent of severe mucositis score, calculated over time either as the AUC or as the average of the three highest values, produce scores that are reproducible and responsive to change.

For clinical purposes, a scoring system based on absolute citrulline values seems practical for determining intestinal MBI. The level of citrulline following MA chemotherapy may help select those patients who will benefit from parenteral nutrition as is the case for those with villous atrophy, where a citrulline level below 10 μmol/L (hypocitrullinaemia) is highly predictive for the need of parental nutrition whereas a level above 10 μmol/L will allow weaning off of parenteral nutrition.\textsuperscript{11,18} Furthermore, we have shown low citrulline concentrations to be associated with bacteraemia,\textsuperscript{26} which could indicate that extra measures should be taken, for example more intensive monitoring of vital signs and temperature registration. The duration of citrullinaemia below a certain threshold might also prove useful for grading the severity of intestinal MBI as is now the cases with grade 4 neutropenia of less than 7–10 days for which the course of antibiotic therapy is shorter than for those with a more protracted duration.\textsuperscript{27}

For intestinal MBI it is conceivable that parenteral nutrition should only be given to patients receiving regimens that induce a long duration of hypocitrullinaemia since parenteral nutrition can promote villous atrophy, increase intestinal permeability and enhance bacterial translocation. Furthermore knowledge of the expected duration of hypocitrullinaemia may help clarify when cytoprotective drugs are necessary and also when antimicrobial therapy should be initiated.

An assessment-score based on circulating citrulline concentrations offers a promising approach to study the relationship between intestinal MBI and post-transplant complications in general including GvHD. Further studies are necessary to explore the predictive value of citrulline for individual patients and to define suitable cut-off values. It is likely, that a citrulline-based as-
ssessment-score could also be of help in the development of successful preventive interventions of agents such as interleukin11 and keratinocyte growth factor that ameliorate GI MBI.\textsuperscript{28,29} Patients treated with reduced intensity regimens, those being treated for solid tumours or with radiotherapy may also benefit from the availability of a better tool for measuring intestinal MBI.

**CONCLUSION**

Circulating citrulline concentrations are able to discriminate between the extent and duration of intestinal mucosal damage induced by different high dose chemotherapy regimens in the clinical setting. A citrulline-based assessment score should be considered the first choice for measuring and monitoring intestinal damage following myeloablative chemotherapy.

**REFERENCES**


CHAPTER 5

CITRULLINE AND ALBUMIN AS BIOMARKERS FOR GASTROINTESTINAL MUCOSITIS IN RECIPIENTS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

WJFM van der Velden, AHE Herbers, RJM Brüggemann, T Feuth, JP Donnelly and NMA Blijlevens.
Bone Marrow Transplant 2013; 48(7): 977-981.
INTRODUCTION
Intestinal mucosal barrier injury (MBI) is a common side effect of intense chemotherapy to prepare patients for hematopoietic stem cell transplantation (HSCT). Measuring intestinal damage objectively remains difficult and clinicians often rely on albumin levels as an indicator of intestinal MBI, but citrulline might be a more specific marker, which has in the past been shown to correlate with clinical signs of intestinal MBI.

PATIENTS AND METHODS
We evaluated the course of albumin and citrulline following different conditioning regimens for HSCT and studied their relatedness to the subsequent inflammatory response using C-reactive protein.

RESULTS
Patterns of albumin and citrulline differed significantly between myeloablative and non-myeloablative conditioning regimens. After myeloablative regimens, decreasing citrulline levels preceded the occurrence of inflammation unlike albumin levels, which decreased thereafter. Albumin levels were greatly influenced by inflammation, confirming it to be a “negative acute-phase protein”, whereas citrulline levels were not.

CONCLUSION
Citrulline appeared a better biomarker of intestinal MBI than did albumin. Measuring citrulline might prove useful in clinical decision making, in identifying intestinal MBI, and it would also be of interest to see how it compares with other biomarkers in the setting of acute intestinal graft-versus-host disease.
INTRODUCTION

Oral and gastrointestinal mucosal barrier injury (MBI) are common side effects of conditioning therapy for hematopoietic stem cell transplantation (HSCT). The degree of MBI is determined by the nature and intensity of the chemotherapy and is associated with adverse clinical and economic outcomes. For example, intestinal MBI results in abdominal pain and diarrhea, increases the risk of infection, and plays a role in graft-versus-host disease (GvHD).

Intestinal MBI is a manifestation of tissue damage to the intestinal mucosal barrier, and several tools have been employed to assess and grade the extent of damage induced by cytotoxic therapy. These include scoring the frequency and volume of diarrhea, measuring oral caloric intake, determining sugar permeability and $^{51}$Cr-EDTA absorption. More recently, it has been shown that the concentration of citrulline in serum and plasma determines the degree of intestinal damage in conditions that are accompanied by intestinal failure. Citrulline concentrations reflect the enterocyte mass and are not influenced by the presence of inflammation.

Citrulline levels have also been shown to be an objective, reproducible, and reliable means of determining intestinal MBI in the setting of HSCT, with low citrulline levels correlated with the clinical scores of intestinal toxicity including diarrhea, and even seemed more reliable as stool volumes and frequency were influenced by oral intake and the use of drugs like opioid analgesics. Citrulline levels were related to the occurrence of bacteremia, the post-conditioning inflammatory response, as well as acute GvHD. Citrulline might be also useful as a predictor of inflammation as its decline usually precedes elevations in C-reactive protein (CRP). However in practice, physicians tend to rely on noting the presence of diarrhea. This is not specific and can have several causes besides intestinal mucositis. Moreover we showed that citrulline and a daily gut score, that included diarrhea, corresponded well, but measuring the amino acid allowed better monitoring of the progress of gut dysfunction as well as its extent.

None the less we wished to see whether serum albumin could also be used to monitor the toxicity resulting from chemotherapy, as this is determined routinely and is more familiar to clinicians. Despite its widespread use there are few reports on the clinical usefulness of monitoring albumin. Moreover, several factors can contribute to hypoalbuminemia of the HSCT recipient, including the underlying disease, nutritional status, occurrence of acute and chronic inflammation, as well as protein-losing enteropathy resulting from MBI. There is also evidence that albumin is, in fact, a “negative acute-phase protein” as low concentrations correlate strongly with body temperature, elevated inflammatory markers including (CRP), interleukin-6 and tumor necrosis factor-α and infection. Indeed serum albumin concentrations inform us about the general state of health and inflammatory status of HSCT recipients, but we wished to explore its value for assessing intestinal mucositis per se. Consequently we evaluated the course of albumin and citrulline following conditioning treatment to prepare for a HSCT, studied their relatedness to the inflammatory response and post-conditioning intestinal damage, and investigated which marker is more valuable for assessing intestinal MBI.
PATIENTS AND METHODS

STUDY POPULATION AND CONDITIONING
Forty-eight (48) patients who had received an autologous and 58 receiving an allogeneic HSCT were included in this analysis. Data had been collected prospectively in all these patients, as they had participated in supportive care trials performed at our hematology department. Patients had given their informed consent to the prospective collection of data and plasma samples for investigational use. The local ethics committee (CMO Regio Arnhem-Nijmegen) approved these studies.

Demographic data and the conditioning regimens are depicted in Table 1 and 2 respectively. All patients had been treated according to the same protocol. All patients receiving myeloablative conditioning were managed with a central venous catheter. In those patients total parental nutrition commenced when patients had insufficient oral intake for 48 hours or more. Gut infections had been excluded in patients included in this analysis.

The sampling had been performed from the start of conditioning (day 1, Figure 1) until discharge. Due to differences in length of stay the period of sampling was different amongst conditioning regimen, being 18, 22, 30, 25 and 22 days for HDM, BEAM, Ida-Cyclo-TBI, Cyclo-ATG-TBI, and Cyclo-Flu, respectively. Because the conditioning started 12 days before HSCT in Ida-Cyclo-TBI conditioned patients the follow-up was considerably longer. The period of sampling ended in most patients at full engraftment, that is on average 14 days post-HSCT. The period did not include episodes with acute GvHD, which occurred later. CRP had been measured daily and albumin twice weekly and samples of plasma had been collected thrice weekly and stored at -80°C for later determination of citrulline. Planned data collections were achieved most of the time, with no more than 5% missing samples (CRP 93/2328 (4%), albumin 36/699 (5%), and citrulline 48/1054 (4.5%).

Unfortunately, clinical data on intestinal mucositis, in this case diarrhea, were not available for all patients. However as mentioned earlier studies have shown citrulline levels to correlate with clinical gut toxicity and an even more objective and reliable marker than measuring diarrhea.10
Table 1. Demographics characteristics of patients, stem cell transplantation and GvHD for each conditioning regimen

<table>
<thead>
<tr>
<th>Conditioning regimen</th>
<th>HDM (N=30)</th>
<th>BEAM (N=18)</th>
<th>Ida-Cyclo-TBI (N=21)</th>
<th>Cyclo-ATG-TBI (N=26)</th>
<th>Cyclo-Flu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), years</td>
<td>53 (35-64)</td>
<td>49 (21-65)</td>
<td>46 (18-64)</td>
<td>44 (22-58)</td>
<td>54 (39-65)</td>
</tr>
<tr>
<td>Gender: M/F</td>
<td>35/21</td>
<td>16/2</td>
<td>12/9</td>
<td>18/8</td>
<td>9/2</td>
</tr>
<tr>
<td>Diagnoses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- MM</td>
<td>30 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- NHL/CLL</td>
<td>-</td>
<td>18 (100%)</td>
<td>-</td>
<td>11 (42%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>- AML</td>
<td>-</td>
<td>-</td>
<td>4 (19%)</td>
<td>2 (10%)</td>
<td>-</td>
</tr>
<tr>
<td>- ALL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4 (19%)</td>
<td>-</td>
</tr>
<tr>
<td>- MDS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- CML/MPD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type of conditioning</td>
<td>MA</td>
<td>MA</td>
<td>MA</td>
<td>MA</td>
<td>NMA</td>
</tr>
<tr>
<td>Type of HSCT</td>
<td>Autologous</td>
<td>Autologous</td>
<td>Matched sibling allogeneic</td>
<td>Matched unrelated allogeneic</td>
<td>Matched sibling allogeneic</td>
</tr>
<tr>
<td>aGvHD, N (%)</td>
<td></td>
<td></td>
<td>10 (48%)</td>
<td>9 (35%)</td>
<td>11 (45%)</td>
</tr>
<tr>
<td>- Grade 1-4</td>
<td>NA</td>
<td>NA</td>
<td>7 (33%)</td>
<td>7 (27%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>- Grade 2-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset GvHD from day of HSCT, mean (95%CI)</td>
<td>NA</td>
<td>NA</td>
<td>28 (19-36)</td>
<td>46 (29-63)</td>
<td>40 (21-61)</td>
</tr>
</tbody>
</table>


Table 2. Conditioning regimen used for the preparation of autologous and allogeneic HSCT

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Doses</th>
<th>Freq.</th>
<th>Days</th>
<th>Type of conditioning</th>
<th>Type of HSCT, day</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDM (N=30) Melphalan</td>
<td>100 mg/m²</td>
<td>od</td>
<td>1, 2</td>
<td>MA</td>
<td>Autologous, day 4</td>
</tr>
<tr>
<td>BEAM (N=18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carmustine</td>
<td>300 mg/m²</td>
<td>od</td>
<td>1</td>
<td>MA</td>
<td>Autologous, day 7</td>
</tr>
<tr>
<td>Etoposide</td>
<td>100 mg/m²</td>
<td>bd</td>
<td>2-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine</td>
<td>100 mg/m²</td>
<td>bd</td>
<td>2-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>140 mg/m²</td>
<td>od</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ida-Cyclo-TBI (N=21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idarubicine</td>
<td>42 mg/m²</td>
<td>In 48h</td>
<td>1</td>
<td>MA</td>
<td>Allogeneic Matched related donor, day 13</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>60 mg/kg</td>
<td>od</td>
<td>7, 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>4.5 Gy</td>
<td></td>
<td>11, 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclo-ATG-TBI (N=26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>60 mg/kg</td>
<td>od</td>
<td>1, 2</td>
<td>MA</td>
<td>Allogeneic Matched unrelated donor, day 9</td>
</tr>
<tr>
<td>Thymoglobuline</td>
<td>2 mg/kg</td>
<td>od</td>
<td>3-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>4.5 Gy</td>
<td></td>
<td>7, 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclo-Flu (N=11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1200 mg/m²</td>
<td>od</td>
<td>1-4</td>
<td>NMA</td>
<td>Allogeneic Matched related donor, day 7</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>30 mg/m²</td>
<td>od</td>
<td>1-4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ATG = anti-thymocyte globulin, Gy = gray, od = once daily, bd = two times daily, MA = myeloablative, NMA = non-myeloablative, TBI = total body irradiation
CRP, ALBUMIN AND CITRULLINE

Hypoalbuminemia was defined according to the CTC version 3.0 criteria by any concentration below 30 g/L. CRP concentrations ≥ 50 mg/L were considered a significant rise from baseline (normal value < 5 mg/L). CRP was chosen over cytokines as measurements are rapid, cheap, and easy, and CRP levels are correlates with pro-inflammatory cytokines such as IL-6 and IL-8. The time course of events was related to the start of conditioning therapy, designated day 1. Citrulline concentrations were determined by a standard procedure for determining amino-acids using HPLC with mass-spectometry detection. Citrulline levels below 10 μmol/L were deemed to indicate hypocitrullinemia, and regarded as reflecting severe intestinal MBI.

STATISTICAL ANALYSIS

We graphically described the development over time of three outcome measures (citrulline, CRP, and albumin) in four conditioning regimen groups (HDM and BEAM were analyzed together). We calculated for each day the mean of the observed values and showed the course over time of these means by connecting the values with straight line segments. Missing values were dealt with by using all data points and applying the Loess smoother with smoothing parameter of 30%. At each point in the dataset a straight line was fitted to a subset of the data consisting of the 30% nearest neighbors with respect to the time axis. This regression line was fitted using weighted least squares giving more weight to points near the point being estimated and less weight to points further away. The value of the regression function for the point was then obtained by evaluating the local regression line using the value of time after transplant for that data point. The fit was complete after regression function values had been computed for all data-points.

We defined three types of events; citrulline < 10 μmol/L, CRP ≥ 50 mg/L, and albumin ≤ 30 g/L, and distinguished between six possible patterns of first occurrences of these events. We evaluated the differences in distribution of patterns between four conditioning regimen groups using Fisher’s exact test or, if needed, a Monte-Carlo approximation using 10,000 samples.

RESULTS AND DISCUSSION

TIME COURSE OF ALBUMIN AND CITRULLINE AFTER MYELOABLATIVE CONDITIONING

Sixty-nine patients received a myeloablative (MA) conditioning regimen to prepare for an autologous HSCT (HDM/BEAM) and matched related donor allogeneic HSCT (Ida-Cyclo-TBI). The pattern of citrulline, CRP and albumin is shown in Figure 1A and B. Early after the start of conditioning there was a rapid decline in citrulline concentrations which reached a level below 10 μmol/L on day 10. This converged with the start of a systemic inflammatory response shown by a rise in CRP concentrations starting around day 10-11 reaching 50 mg/L by day 12. Consequently, the decline in citrulline preceded the inflammatory response, indicating that GI...
damage induces the inflammatory response seen after conditioning.\textsuperscript{12} The course of albumin levels was different with the gradual descent lagging behind that of citrulline (Figure 1A and B) reaching levels of ≤ 30 g/L on day 13, hence 3 days after the nadir of citrulline had been reached and after the inflammatory response had commenced. This suggests that hypoalbuminemia did not precede but rather followed the occurrence of inflammation induced by GI damage.

Figure 1. Citrulline, CRP and albumin values of the different conditioning regimen.

A = HDM and BEAM, B = Ida-Cyclo-TBI, C = Cyclo-ATG-TBI, D = Cyclo-Flu. Days from the start of conditioning. Black lines = observed mean values, Red lines = loess smoothed means. Thresholds depicted in the figure are 10 μmol/L, 50 gr/L, and 30 gr/L for citrulline, CRP, and albumin, respectively.
Chapter 5

TIME COURSE OF ALBUMIN AND CITRULLINE AFTER MYELOABLATIVE CONDITIONING CONTAINING ANTI-THYMOCYTE GLOBULIN

The course of events was different for the group of 21 patients who had received ATG in their MA-regimen for a matched unrelated donor (MUD) HSCT. The course of citrulline was similar, but now albumin levels declined with levels approaching 30 gr/L already on day 6 (Figure 1C). This coincided with an inflammatory response (day 3) resulting from cytokine-release induced by ATG which resulted in the development of fever in most patients. Hereafter the decline in albumin halted and levels remained rather constant until day 12, following which, there was a progressive decline as seen in the other MA regimens coinciding with the inflammation resulting from intestinal damage producing a second peak of CRP from day 11-12 (Figure 1C).

TIME COURSE OF ALBUMIN AND CITRULLINE AFTER NON-MYELOABLATIVE CONDITIONING

Only 11 patients had received a non-myeloablative (NMA) regimen. Nevertheless less damage was expected as the chemotherapy was less mucotoxic and was reflected in a limited and short-lived decline in citrulline levels with only a few patients experiencing hypocitrullinaemia. There was no inflammation attributed to intestinal damage. An early modest rise in CRP in some cases coincided with the administration of chemotherapy and the second rise in CRP was mostly late occurring during engraftment reaching modest levels below 50 mg/L. Four patients had a significant rise above 50 mg/L; two during engraftment, one due to a thrombophlebitis and another due to pneumonia. The decline in albumin levels was less pronounced although most patients reached levels below 30 g/L, but in contrast with what was seen for MA regimens, this did not occur simultaneously with a rise in CRP (Figure 1D).

ALBUMIN AND CITRULLINE LEVELS ARE NOT EQUAL AS BIOMARKERS OF INTESTINAL MBI

Additional statistical analysis confirmed the observed differences in the pattern of post-conditioning events amongst the groups (Figure 1, Monte Carlo approximation of Fisher exact test, \( P <0.0001 \)). There was no difference in patterns of events between groups A and B (\( P = 0.42 \)), but there were differences in pattern between C and A/B, D and A/B, and C and D (with \( P \) values based on Fisher exacts tests: <0.0001, 0.006, and 0.003, respectively). These differences are further illustrated graphically in Figure 2. Following HDM/BEAM and Ida-Cyclo-TBI, citrulline levels declined before or at the time CRP levels became elevated and albumin levels declined thereafter. Following ATG-Cyclo-TBI, a regimen considered equally mucotoxic, the course of albumin but not citrulline was very different. The first peak in CRP levels preceded a decline in albumin levels, which was then followed by a second peak in CRP levels that coincided with the development of hypocitrullinaemia and a further decline in albumin levels. Following Cyclo-Flu the attenuated GI damage precluded the marked hypocitrullinaemia and the corresponding inflammation in most cases. Albumin levels did not correspond to citrulline levels as these were already rising whilst albumin levels were decreasing. Also the relationship between the occurrence of hypoalbuminemia and inflammation was less clear.
Figure 2. Comparison of regression lines of citrulline, CRP and albumin amongst the conditioning regimen groups

A = HDM and BEAM, B = Ida-Cyclo-TBI, C = Cyclo-ATG-TBI, D = Cyclo-Flu. The course of citrulline, C-reactive protein and albumin differed statistically amongst the different conditioning regimens (P < 0.01)
Hence it seems fair to conclude that albumin and citrulline levels are not equal with regard to measuring intestinal MBI. Citrulline follows a distinctive course that is clearly different for MA and NMA regimens which is almost certainly due to the mucotoxicity induced. The variation in citrulline concentrations was also less and the course of levels more consistent than was found for albumin. Moreover citrulline can be considered a more reliable marker of intestinal damage because it is a very specific biomarker of enterocyte mass.\textsuperscript{5,9} By contrast, albumin levels appear mainly influenced by the occurrence of inflammation that resulted either from the host response to intestinal damage, the occurrence of infection, or to the cytokines released by the conditioning therapy particularly that containing ATG. The decline in albumin levels seen was not likely the result of malnutrition as hypoalbuminemia occurred early after the start of conditioning therapy when the food intake is still adequate for most patients.\textsuperscript{21} Dynamic changes in albumin levels are mostly due to changes in catabolic rate and extra-vascular shift (capillary leakage) and not to malnutrition as albumin has an estimated half-life of 20 days.\textsuperscript{21-23} Albumin decline after day 12 might be the result of the loss of proteins from the damaged intestinal tract as has been suggested for GvHD, and not solely from extra-vascular shift (capillary leakage) seen with inflammation. Nevertheless, the decline occurred late, sometime after intestinal damage and the related inflammatory response had already occurred. Hence hypoalbuminaemia following HSCT might be better regarded as a “negative acute-phase response” or a late sequel of intestinal MBI that comes with protein loss. Whichever is the case, citrulline levels appear a better biomarker of intestinal damage resulting from chemo- and radiotherapy than albumin.

Since citrulline is a better biomarker of intestinal MBI, and may even be a suitable surrogate marker, it would be of particular interest to see how useful a biomarker it is to score and measure GvHD that involves the intestinal tract. Albumin has been shown to be a biomarker of gastrointestinal GvHD, as it correlates with its severity\textsuperscript{24,25}, but this might be confounded by the inflammatory response that occurs with GvHD.

**CONCLUSION**

Citrulline provides a better biomarker of chemotherapy and radiotherapy-induced intestinal MBI, than does albumin. Determining citrulline levels might prove useful in helping decide whether and when therapeutic and nutritional interventions are necessary for patients treated with intensive chemotherapy and radiotherapy.
REFERENCES


18. Schots R, Van R, Othman TB et al. An early increase in serum levels of C-reactive protein is an independent risk factor for the occurrence of major complications and 100-day transplant-related mortality after allogeneic bone marrow transplantation. Bone Marrow Transplant 2002; 30: 441-446.


PART III

FEBRILE NEUTROPENIA
OR
FEBRILE MUCOSITIS?
CHAPTER 6

BACTERAEMIA COINCIDES WITH LOW CITRULLINE CONCENTRATIONS AFTER HIGH-DOSE MELPHALAN IN AUTOLOGOUS HSCT RECIPIENTS

ABSTRACT

INTRODUCTION
Mucosal damage to the intestines induced by myeloablative (MA) conditioning for allogeneic hematopoietic stem cell transplantation (HSCT) can be determined by the concentration of citrulline, which is a functional marker of small intestinal enterocytes. Low citrulline concentrations in blood coincide with and are a response to severe mucosal barrier injury. The aim of this study was to explore the kinetics of plasma citrulline concentrations in patients receiving high-dose melphalan 200 mg/m² (HDM), given in two days to prepare for an autologous HSCT and to determine the relationship between these levels and bacteraemia.

PATIENTS AND METHODS
Plasma samples from 29 patients were collected, starting before the MA regimen and 3 times per week thereafter until discharge.

RESULTS
The average citrulline concentration at baseline was 27.6 μmol/L ± 4.0 (mean ± 95% confidence interval (CI)) and concentrations declined rapidly thereafter reaching a nadir averaging 6.7 μmol/L ± 2.7, 12 days after starting HDM. Citrulline concentrations, only increased gradually and were still low (12 μmol/L ± 4) at discharge. A total of 20 patients developed fever, which was associated with bacteraemia in 10 cases. Their mean citrulline concentrations were lower (5.5 μmol/L ± 1.5) than were those of patients without bacteraemia (10.2 μmol/L ± 3.9). Importantly, neither the number of preceding neutropenic days nor the mean C-reactive protein (CRP) concentration at the onset of fever was different between these two groups.

CONCLUSION
Citrulline concentrations rapidly decline after HDM reflecting intestinal mucosal barrier injury. Low citrulline, rather than the duration of neutropenia, is associated with bacteraemia indicating the importance of an intact mucosal barrier in neutropenic patients.
INTRODUCTION

Mucositis is the clinical manifestation of mucosal barrier injury (MBI) and is the most frequent cause of morbidity associated with myeloablative (MA) treatment to prepare for hematopoietic stem cell transplant (HSCT). Impaired integrity of the mucosal barrier induced by anticancer therapy is thought to promote translocation of micro-organisms from the lumen of the digestive tract to the blood stream resulting in bacteraemia. Whereas oral MBI is relatively easy to recognise, detection of intestinal mucosal injury is more demanding. Recently, it was shown that citrulline appeared to be particularly useful to detect intestinal damage, as blood concentrations of this amino acid directly reflect functioning small intestinal cell mass. Furthermore, low plasma concentrations of citrulline coincided with the MBI of the small intestine induced by the intensive MA therapy used to prepare for a T-cell depleted allogeneic hematopoietic stem cell transplant (HSCT). These levels also corresponded to the severity and extent of gut injury. We explored the kinetics of citrulline concentrations in a cohort of patients treated with 200 mg/m² melphalan, given in two days (HDM), to prepare for an autologous HSCT. Furthermore, we determined the relationship of citrulline concentrations to bacteraemia, as the risk is known to be increased by MBI.

PATIENTS AND METHODS

PATIENT MANAGEMENT

From May 2004 to May 2005, 29 consecutive patients (12 female; 17 male) with a mean age of 55 years (range 30 - 65) were admitted to undergo an autologous HSCT to treat multiple myeloma (MM). Each patient was managed with a triple-lumen central venous catheter (CVC) and received melphalan at a dose of 100 mg/m² on days −3 and −2 before transplant. Each patient received parenteral nutrition from the day of transplant onwards and ondansetron as an antiemetic. Anti-infective prophylaxis consisted of ciprofloxacin and valaciclovir. Fluconazole was given only to patients who were colonised by Candida albicans. No hematopoietic growth factors or keratinocyte growth factors were used.

DEFINING BACTERAEMIA

At the onset of fever (single axillary temperature of ≥ 38.5 °C) during neutropenia (neutrophil count of ≤ 0.5 x 10⁹/l ), a single 10 ml sample of blood was obtained from each lumen of the central venous catheter (CVC) and inoculated into an aerobic BACTEC plus (BD Diagnostic System, Sparks, USA) blood culture bottle. A further two 20 ml samples of blood were drawn from separate peripheral veins and were each inoculated equally into a blood culture set comprising an aerobic and anaerobic BACTEC plus culture bottle. Ceftazidime was administered immediately afterwards. Bacteraemia was defined by any single blood culture yielding bacteria except for...
coagulase-negative staphylococci (CoNS), which required the same strain to be recovered from two separate cultures. C-reactive protein (CRP) was measured daily until discharge using turbidimetric immunoassay (Aeroset©, Abbott).

CITRULLINE ANALYSIS
Blood was collected in heparin from each patient through the CVC before starting therapy and on each Monday, Wednesday, and Friday thereafter until discharge. Plasma was prepared and stored at –80°C for later analysis. Citrulline concentrations (μmol/L) were measured by a standard procedure for determining amino acids using high-performance liquid chromatography (Shumadzu©).6

ORAL MBI ASSESSMENT
Oral MBI was scored daily by trained nurses who recorded the presence of pain, lesions, erythema, oedema, bleeding, dryness and of viscous mucus allocating a score of 0 for normal to 3 for severe.7 The separate values were summed up to yield a daily oral mucositis score (DMS).

GASTROINTESTINAL (GI) MBI ASSESSMENT
GI MBI was determined from the frequency of vomiting and diarrhoea, the occurrence of nausea, abdominal complaints, faecal incontinency and the volume of diarrhoea. Each item was scored from 0 (normal) to 3 (severe) and the values were summed to yield the daily gut score (DGS).1 All gut toxicities developed following chemotherapy were attributed to MBI unless there was a more plausible alternative cause such as an adverse drug reaction or a proven infection.

DATA ANALYSIS
SPSS for windows software (release 12.1) was used for statistical analysis. All results are expressed as the mean and 95% confidence interval (CI). The time course of citrulline was estimated from the day cytotoxic therapy was given (day 1).8 The number of days with neutropenia before the onset of fever was counted and the DMS, DGS, the concentrations of citrulline and CRP, and the absolute leukocyte and neutrophil count on that day were noted. Missing data were replaced with the values for the day before or after the onset of fever. A two-sided unpaired Student’s t-test was used to calculate significant differences with respect to baseline. Analysis of variance was used to test differences between and within groups. A P-value of < 0.05 was considered statistically significant.
RESULTS

All patients had an estimated renal creatinine clearance above 50 ml/L at admission using the Cockcroft & Gault formula, and no adjustments were made to the dose of melphalan. None of the patients developed an invasive fungal disease and all survived the first 6 weeks after transplant.

The average citrulline concentration (mean ± 95% CI) at baseline for all patients was 27.6 μmol/L ± 4.0 and concentrations declined rapidly thereafter reaching a nadir averaging 6.7 μmol/L ± 2.7 12 days after starting HDM (on day +8 of HSCT) (Figure 1). Citrulline concentrations then increased gradually but were still markedly low with a mean of 12 μmol/L ± 4 at discharge.

Figure 1. Time course of serum citrulline concentration (μmol/L)

All patients became profoundly neutropenic (mean onset 8.5 ± 0.3 days after starting HDM) with a mean duration of neutropenia of 10.2 days ± 2.7. 20 patients (69%) developed fever which was accompanied by bacteraemia in 10 cases, five of which were due to a single species
(two *Streptococcus mitis*, two *Staphylococcus epidermidis* and one *Staphylococcus haemolyticus*) and five due to two species (one case each of *Streptococcus mitis* & *Staphylococcus epidermidis*, *Streptococcus mitis* & *Staphylococcus hominis*, *Streptococcus mitis* & *Staphylococcus capitis*, *Staphylococcus epidermidis* & *Staphylococcus hominis* and *Staphylococcus epidermidis* & *Staphylococcus saprophyticus*). Cultures drawn from peripheral and from central lines yielded the same bacteria. The mean citrulline level at onset of fever among patients with bacteraemia was 5.5 μmol/L ± 1.5 and was significantly lower than that of patients without bacteraemia (10.2 μmol/L ± 3.9) (P < 0.05). However, there was no difference in the time to onset of fever from neutropenia, in CRP concentrations, leukocyte counts, DGS or DMS on the day of fever between the groups (Table 1). Neither was a difference found between the mean baseline citrulline concentrations, which were, respectively, 23.0 μmol/L ± 3.5 for patients with bacteraemia compared with 31.3 μmol/L ± 3.4 for those without bacteraemia.

**Table 1.** Mean ± 95% CI of citrulline concentrations, oral mucositis (DMS), gut toxicity (DGS), CRP and leukocytes on first day of fever and total neutropenic days before onset of fever in patients with neutropenic fever with or without bacteraemia

<table>
<thead>
<tr>
<th>On first day of fever (N=20) (mean ± 95%CI)</th>
<th>Bacteraemia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>present (N=10)</td>
<td>absent (N=10)</td>
</tr>
<tr>
<td>Citrulline (μmol/L)</td>
<td>5.5 ± 1.5</td>
<td>10.2 ± 3.9</td>
</tr>
<tr>
<td>DMS</td>
<td>5.3 ± 1.6</td>
<td>6.0 ± 2.3</td>
</tr>
<tr>
<td>DGS</td>
<td>4.3 ± 1.2</td>
<td>3.8 ± 1.1</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>63 ± 33</td>
<td>56 ± 31</td>
</tr>
<tr>
<td>Leucocytes (x10^9/l)</td>
<td>0.1 ± 0.03</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>Neutropenic days before the onset of fever</td>
<td>4.0 ± 1.1</td>
<td>3.6 ± 1.2</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; CRP = C-reactive protein; DGS = daily gut score; DMS = daily oral mucositis score; ns = not significant.

**DISCUSSION**

The plasma concentrations of citrulline for all patients reached a nadir within 12 days after starting myeloablative therapy indicating intestinal mucosal damage, with the lowest citrulline concentrations coinciding with the onset of bacteraemia. Patients with bacteraemia had significantly lower citrulline concentrations on the first day of fever than did those without
bacteraemia. But there was no difference in the total days before the onset of fever after patients became neutropenic. This suggests that the severity of gut MBI determines whether bacteraemia occurs or not rather than neutropenia per se.

Neutropenia (granulocytes ≤ 0.5 x 10⁹/l) has been used for 40 years to recognise those patients who are at imminent risk of developing infectious complications following intensive chemotherapy. This formed the foundation for developing a successful strategy for managing these patients, namely administering broad-spectrum antimicrobial therapy promptly as soon as fever occurs during neutropenia. Indeed, empirical antibacterial therapy is still the backbone of the supportive care given to these patients. However, it has become clear that MBI plays a distinct role in determining the outcome of HSCT. With mucosal damage being indicated by declining citrulline levels, we may be able to identify more effectively the risk period for developing bacteraemia in neutropenic patients. Of the 29 patients we investigated, 20 became febrile but only half of them developed bacteraemia and their citrulline concentrations were lower than those found in the other patients. Moreover, these concentrations correspond to diffuse total villous atrophy.

Interestingly, the maximum World Health Organization scale grade of oral MBI was also shown to occur on day 12 after starting cytostatic chemotherapy. In our study, neither DMS nor DGS were significantly different between those with and without bacteraemia, confirming that these instruments measure different features of digestive tract injury compared to citrulline concentrations.

We also found that CRP was not useful for detecting or predicting bacteraemia as has been noted by others. Most likely, this is because CRP marks a response to a variety of stimuli other than infection and its elaboration is relatively slow. However, the time course of CRP is relevant. A study with rats treated with 5-fluorouracil (5-FU) showed that the release of pro-inflammatory cytokines was associated with evolving gastrointestinal (GI) MBI and preceded bacterial translocation. Consequently, an increase in CRP levels may reflect the severity of GI MBI.

All the episodes of bacteraemia that we observed were due either to coagulase-negative staphylococci (CoNS) or oral viridans streptococci (OVS). This is most likely a direct consequence of giving ciprofloxacin as prophylaxis, which effectively prevents bacteraemia due to Gram-negative bacilli, while giving selective advantage to the less susceptible Gram-positive bacteria. The blood cultures of 5 of the 10 patients with bacteraemia yielded two bacterial species which might simply reflect greater sampling efficiency, as we obtained a total of 70 ml of blood for blood cultures, which is more than others report.

OVS are primarily oral commensal flora; however, molecular analysis of the microbiota in the stomach shows these bacteria can be found in large numbers along with Helicobacter pylori. In children treated for acute myeloid leukaemia (AML), there was an association between the presence of GI toxicity and the development of OVS bacteraemia. Similarly, CoNS have been assumed to originate from central venous catheters. However, there is a growing body
of evidence that these bacteria may, in fact, also originate from the GI tract. Indeed, molecular analysis of CoNS isolated from blood cultures indicated that the mucosa was the origin in most of the cases. Moreover, bacteraemia due to these staphylococci has been shown to occur mostly within the first 2 weeks after transplant when gut integrity is markedly disturbed. A recent study reported a threefold increase in documented infections in patients with GI MBI, though there was no relationship between MBI and the depth or duration of neutropenia. Our results concur suggesting that MBI is an independent risk factor for the bacterial translocation that precedes bacteraemia. A small study of Ellis et al. showed treatment with recombinant human Interleukin-11 resulted in less bacteraemia and, although a protective effect on the mucosa was not observed, gut permeability improved. Hence, agents such as recombinant human Interleukin-11 and the keratinocyte growth factor palifermin, which are designed to protect the mucosa, may prove helpful in reducing bacterial infection in neutropenic patients. Indeed, in the recent study of palifermin, there were fewer episodes of bacteraemia among those given the drug than was found among those given placebo (15 versus 25%). However the difference was not statistically significant and no details were provided on the aetiology of bacteraemia.

Severe disruption of the mucosal barrier is clearly not the only risk factor for developing bacteraemia, which affected only a third of our patients with low citrulline concentrations. To identify those patients at risk for bacteraemia, citrulline measurements need to be combined with other tests. For instance, the Multinational Association of Supportive Care of Cancer (MASCC) developed a risk-score to predict at the onset of fever during neutropenia which patients are at high risk for development of serious medical complications. Endothelial cells, like the epithelial cells of the gut, play an important part in the innate immune system in neutropenic patients and produce interleukin-8 when exposed to bacteria. Hence, perhaps monitoring interleukin-8 levels combined with the MASCC risk score, when patients have low citrulline concentrations, might help distinguish those at risk of developing bacteraemia from those who are not.

CONCLUSION

This study provides more evidence that the damage induced by cytostatic chemotherapy to the mucosa plays an important role in the morbidity and mortality after intensive chemotherapy. Preventing or restoring damage to the mucosa may offer a more effective means of preventing bacteraemia after transplant than giving antimicrobial agents. Citrulline may provide added utility in the diagnostic work-up of neutropenic patients to help recognise those who are at increased risk for developing bacteraemia.
REFERENCES


CHAPTER 7

INTESTINAL MBI DETERMINES THE INFLAMMATORY RESPONSE AND EARLY COMPLICATIONS IN PATIENTS RECEIVING CONDITIONING FOR A HSCT

WJFM van der Velden, AHE Herbers, T Feuth, NPM Schaap, JP Donnelly and NMA Blijlevens.

ABSTRACT

INTRODUCTION
Hematopoietic stem cell transplantation (HSCT) is still complicated by the occurrence of fever and inflammatory complications attributed to neutropenia and subsequent infectious complications. The role of mucosal barrier injury (MBI) of the intestinal tract therein has received little attention.

PATIENTS AND METHODS
We performed a retrospective analysis in 163 HSCT recipients of which data had been collected prospectively on intestinal damage (citrulline), inflammation (C-reactive protein), and neutrophil count. Six different conditioning regimens were studied: 5 myeloablative (MA) and 1 non-myeloablative (NMA). Linear mixed model multivariate and AUC analyses were used to define the role of intestinal damage in post-HSCT inflammation. We also studied the relationship between the degree of intestinal damage and the occurrence of early post-HSCT complications.

RESULTS
In the 5 MA regimens there was a striking pattern of inflammatory response that coincided with the occurrence of severe intestinal damage. This contrasted with a modest inflammatory response seen in the NMA regimen in which intestinal damage was limited. With linear mixed model analysis the degree of intestinal damage was shown the most important determinant of the inflammatory response, and both neutropenia and bacteremia had only a minor impact. AUC analysis revealed a strong correlation between citrulline and CRP (Pearson correlation $r = 0.96$). Intestinal damage was associated with the occurrence of bacteremia and acute lung injury, and influenced the kinetics of acute graft-versus-host disease.

CONCLUSION
The degree of intestinal damage after myeloablative conditioning appeared to be the most important determinant of the inflammatory response following HSCT, and was associated with inflammatory complications. Studies should explore ways to ameliorate cytotoxic therapy-induced intestinal damage in order to reduce complications associated with myeloablative conditioning therapy.
INTRODUCTION

Treating patients with hematological malignancies by use of hematopoietic stem cell transplantation (HSCT) is still complicated by the occurrence of infectious and inflammatory complications including sepsis, acute lung injury, and graft-versus-host disease (GvHD). Historically the focus was on neutropenia and fever (“febrile neutropenia”) and its relation to infections. However, a substantial number of HSCT recipients develop fever unrelated to infection (“unexplained fever”), resulting from other causes including cytotoxic therapy-induced mucosal barrier injury (MBI). Many studies have shown associations between the magnitude of the C-reactive protein (CRP) response and cytokine release and post-HSCT complications, and these complications might therefore best be regarded as manifestations of a systemic inflammatory response syndrome (SIRS). Other studies have shown that infections may contribute to non-infectious complications including acute GvHD. However, few if any of these studies addressed the role of MBI per se as an isolated cause of inflammation and risk factor for infections, nor its role in the pathogenesis of inflammatory complications. Animal models have enhanced our understanding of the inflammatory processes that take place in the intestine following chemotherapy, and in the clinical setting of HSCT the relationship between intestinal damage and the inflammatory response has become better appreciated. Mucosal damage and deregulated host-microbial interactions have also been shown to contribute to SIRS and post-HSCT complications such as acute GvHD. Therefore, we hypothesized that intestinal damage could be the most important determinant of early SIRS following conditioning with chemotherapy and radiotherapy and that the degree of damage correlates with the occurrence of post-HSCT complications.

Studying damage to the gastro-intestinal (GI) tract during HSCT remains difficult, since it remains hidden and hitherto only indirect and non-specific tests were available. Measuring serum or plasma citrulline levels provides a more direct and specific way of determining intestinal damage of certain conditions that are accompanied by gut failure. Recently, citrulline-based assessment of intestinal damage has also shown to be objective, reproducible, specific and reliable in the setting of HSCT. To test our hypothesis we studied the relationship between the magnitude of the inflammatory response and the degree of intestinal damage as measured by citrulline, the duration of neutropenia, and the occurrence of bacteraemia. To achieve this we selected recipients of a HSCT for which 5 cohorts of patients had been given different myeloablative (MA) conditioning regimens and a single cohort had received a non-myeloablative (NMA) conditioning regimen. We also investigated whether we could determine a relationship between the degree of intestinal damage and the occurrence of early post-HSCT complications.
PATIENTS AND METHODS

STUDY POPULATION
This was a retrospective analysis of 163 patients who had received an autologous or allogeneic HSCT in our hospital from May 2004 to December 2007. Plasma had been collected prospectively and stored for later analysis of citrulline, but other data including CRP, temperature, and clinical and microbiological infections had been prospectively gathered from the day of starting chemotherapy. Patients had given their informed consent to the prospective collection of data and plasma samples for investigational use. The local ethics committee (CMO Regio Arnhem-Nijmegen) judged that no formal approval for this study was necessary regarding the fact that data were used anonymously and the analysis would not reveal results harming contributing patients.

CONDITIONING REGIMENS AND STEM CELL TRANSPLANTATION
The MA and NMA regimens are depicted in Table 1. All patients received a stem cell graft on the day scheduled. Autologous HSCT grafts contained at least \( 2.0 \times 10^6 \) CD34+ cells per kg, and allogeneic HSCT partially T cell-depleted grafts contained on average \( 3.0 \times 10^6 \) CD34+ cells per kg and \( 0.5 \times 10^6 \) CD3+ cells per kg.

Table 1. Conditioning regimens

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Doses</th>
<th>Freq.</th>
<th>Days</th>
<th>Type of conditioning</th>
<th>Type of HSCT, day</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDM</td>
<td>Melphalan</td>
<td>100 mg/m²</td>
<td>od</td>
<td>1, 2</td>
<td>MA Autologous, day 4</td>
</tr>
<tr>
<td>BEAM</td>
<td>Carmustine (BCNU) Etoside</td>
<td>300 mg/m²</td>
<td>od</td>
<td>1</td>
<td>MA Autologous, day 7</td>
</tr>
<tr>
<td></td>
<td>Cytarabine</td>
<td>100 mg/m²</td>
<td>bd</td>
<td>2-5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan</td>
<td>140 mg/m²</td>
<td>od</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Ida-Cyclo-TBI</td>
<td>Idarubicine</td>
<td>42 mg/m²</td>
<td>in 48h</td>
<td>1</td>
<td>MA Allogeneic Matched related donor, day 13</td>
</tr>
<tr>
<td>Cyclophysphamide</td>
<td>TBI</td>
<td>60 mg/kg 2 4.5 Gy</td>
<td>od</td>
<td>7, 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide Thymoglobuline TBI</td>
<td>60 mg/kg 2 4.5 Gy</td>
<td>od</td>
<td>3-6</td>
<td>MA Allogeneic Matched unrelated donor, day 9</td>
</tr>
<tr>
<td></td>
<td>TBI</td>
<td>60 mg/kg 4.5 Gy</td>
<td>od</td>
<td>1, 2</td>
<td>MA Allogeneic Matched related donor, day 7</td>
</tr>
<tr>
<td></td>
<td>TBI</td>
<td>60 mg/kg 4.5 Gy</td>
<td>od</td>
<td>1, 2</td>
<td>NMA Allogeneic Matched related donor, day 7</td>
</tr>
<tr>
<td></td>
<td>TBI</td>
<td>1200 mg/m² 30 mg/m²</td>
<td>od</td>
<td>1-4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Freq. = frequency, MA = myeloablative, NMA = non-myeloablative, od = once daily, bd = two times daily, TBI = total body irradiation.
TREATMENT PROTOCOL
All patients were treated according to the same protocol, which has been described earlier. GvHD prophylaxis consisted of cyclosporine only. Anti-microbial prophylaxis consisted of ciprofloxacin and valaciclovir. Fever was defined by a single axillary temperature ≥ 38.5 °C. At the onset of fever 40 mL of peripheral blood was obtained for culture together with 10 mL from each lumen of the catheter, patients were examined for any sign of local infection, and empirical therapy with ceftazidime was started. Neutropenia was defined as granulocytes ≤ 0.5 x 10^9/L, and the duration and depth was recorded. CRP levels (mg/L) were determined every day and the maximum CRP (CRP_{max}) recorded. Plasma citrulline was determined to estimate intestinal damage before the start of conditioning and 3 times weekly thereafter until discharge. Citrulline concentrations (μmol/L) were measured by a standard procedure for determining amino acids using high-performance liquid chromatography. Citrulline levels below 10 μmol/L were deemed to indicate hypocitrullinemia, and were regarded as reflecting severe intestinal damage.

DEFINITION OF STEM CELL TRANSPLANTATION COMPLICATIONS
Clinical and microbiologically defined infections were defined according to the Consensus definitions of Immunocompromised Host Society. A blood culture was considered to represent bacteraemia if one or more bottles yielded a microorganism, except in the case of coagulase-negative staphylococci (CoNS), which required recovery of the same strain from two separate positive blood cultures. The incidence of bacteraemia that occurred on the day of fever was documented and compared among the regimens. Invasive fungal diseases were scored according to the EORTC/MSG consensus guidelines. Acute lung injury (ALI) was defined according to current guidelines. Acute GvHD, GvHD occurring the first 100 days after HSCT, was graded according to the criteria of Przepiorka et al. Early mortality related to HSCT complications was defined as any death occurring within 30 days following HSCT (day +30), but unrelated to the underlying disease.

DATA ANALYSIS
We employed descriptive statistics for fever, neutrophil count, CRP levels, and citrulline levels which were expressed as mean values together with the 95% confidence interval (Table 2). As citrulline was measured three times weekly, the real nadir might have been attained between two measurements and hence was likely missed.
### Table 2. General characteristics

<table>
<thead>
<tr>
<th>Conditioning</th>
<th>HDM (N=56)</th>
<th>BEAM (N=21)</th>
<th>Ida- Cyclo-TBI (N=28)</th>
<th>Cyclo-ATG- TBI (N=34)</th>
<th>Cyclo-TBI (N=10)</th>
<th>Cyclo-Flu (N=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), years</td>
<td>56 (33-65)</td>
<td>47 (18-65)</td>
<td>46 (18-64)</td>
<td>43 (20-58)</td>
<td>50 (38-59)</td>
<td>54 (39-65)</td>
</tr>
<tr>
<td>Gender: M/F</td>
<td>35/21</td>
<td>17/4</td>
<td>13/15</td>
<td>21/13</td>
<td>8/2</td>
<td>10/4</td>
</tr>
<tr>
<td>Diagnoses:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>56 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>NHL/CLL</td>
<td>-</td>
<td>-</td>
<td>21 (100%)</td>
<td>13 (38%)</td>
<td>8 (80%)</td>
<td>-</td>
</tr>
<tr>
<td>AML</td>
<td>12 (42.5%)</td>
<td>12 (42.5%)</td>
<td>12 (42.5%)</td>
<td>8 (23.5%)</td>
<td>2 (20%)</td>
<td>-</td>
</tr>
<tr>
<td>ALL</td>
<td>3 (10.5%)</td>
<td>3 (10.5%)</td>
<td>3 (10.5%)</td>
<td>4 (11.75%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MDS</td>
<td>6 (22%)</td>
<td>6 (22%)</td>
<td>6 (22%)</td>
<td>4 (11.75%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CML/MPD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 (15%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type of conditioning</td>
<td>MA</td>
<td>MA</td>
<td>Matched sibling allogeneic</td>
<td>Matched unrelated allogeneic</td>
<td>Matched sibling allogeneic</td>
<td>Matched sibling allogeneic</td>
</tr>
<tr>
<td>Type of HSCT</td>
<td>Autologous</td>
<td>Autologous</td>
<td>Matched sibling allogeneic</td>
<td>Matched unrelated allogeneic</td>
<td>Matched sibling allogeneic</td>
<td>Matched sibling allogeneic</td>
</tr>
<tr>
<td>Fever (axillary temperature ≥ 38.5 °C)</td>
<td>88.0%</td>
<td>90.5%</td>
<td>100%</td>
<td>Early: 73.5% Late: 100%</td>
<td>100%</td>
<td>Early: 28.6% Late: 78.6%</td>
</tr>
<tr>
<td>Fever, day from start conditioning, mean (95%CI)</td>
<td>11.8 (11.4-12.2)</td>
<td>13.0 (12.2-13.9)</td>
<td>12.9 (12.2-13.6)</td>
<td>13.4 (12.0-14.8)</td>
<td>13.4 (12.0-14.8)</td>
<td>3.0 (1.7-4.3)</td>
</tr>
<tr>
<td>Neutrophils ≤ 0.5 x10⁹/L, days (95%CI)</td>
<td>8.4 (8.0-8.7)</td>
<td>9.5 (8.5-10.5)</td>
<td>15.8 (14.6-17.0)</td>
<td>15.5 (14.6-16.4)</td>
<td>11.1 (9.8-12.4)</td>
<td>12.4 (10.8-14.1)</td>
</tr>
<tr>
<td>Citrulline &lt; 10 μmol/L, number of patients, (%)</td>
<td>51 (91%)</td>
<td>21 (100%)</td>
<td>26 (93%)</td>
<td>30 (88%)</td>
<td>10 (100%)</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>Measurements with citrulline &lt; 10 μmol/L, mean (95%CI)</td>
<td>3.5 (3.2-3.9)</td>
<td>4.7 (4.0-5.3)</td>
<td>6.2 (4.6-7.8)</td>
<td>4.8 (4.0-5.7)</td>
<td>3.5 (2.4-4.6)</td>
<td>3.0 (1.1-4.9)</td>
</tr>
<tr>
<td>Observed citrulline nadir μmol/L, mean (95%CI)*</td>
<td>6.0 (5.4-6.6)</td>
<td>4.3 (3.5-5.1)</td>
<td>4.6 (3.9-5.3)</td>
<td>5.6 (4.9-6.3)</td>
<td>5.6 (4.2-7.0)</td>
<td>10.8 (8.9-12.6)</td>
</tr>
<tr>
<td>Citrulline &lt;10 μmol/L, days, mean (95%CI)&amp;#</td>
<td>7.9 (7.1-8.7)</td>
<td>11.2 (9.6-12.9)</td>
<td>17.7 (15.6-19.8)</td>
<td>14.6 (13.3-15.9)</td>
<td>11.0 (7.9-14.1)</td>
<td>7.5 (5.4-9.6)</td>
</tr>
<tr>
<td>Citrulline nadir μmol/L, mean (95%CI)&amp;</td>
<td>6.5 (5.7-7.2)</td>
<td>4.9 (3.7-6.0)</td>
<td>4.5 (3.5-5.7)</td>
<td>7.0 (6.1-7.9)</td>
<td>6.6 (5.1-8.1)</td>
<td>12.4 (10.2-14.6)</td>
</tr>
<tr>
<td>CRPmax (mg/L), mean (95%CI)</td>
<td>163 (136-189)</td>
<td>202 (160-246)</td>
<td>257 (222-291)</td>
<td>188 (162-213)</td>
<td>211 (154-269)</td>
<td>66 (38-95)</td>
</tr>
</tbody>
</table>

*Only those patients included with citrulline levels below 10 μmol/L. & Based on estimated values.

To compensate for this and study the true length of time in which citrulline levels were below 10 μmol/L we modeled the course of citrulline as a function of time during the first 30 days by developing a linear mixed model using first, second, third and fourth power of time as fixed factors to predict the citrulline levels after taking into account the within-person correlations by incorporating a random patient intercept. To describe the relationship of CRP to the neutrophil count, intestinal damage (citrulline concentration) and bacteraemia we used several linear mixed models for the first 30 days with random patient effect and the logarithmic transformed CRP (log CRP) as the outcome variable.

To assess the impact of conditioning on intestinal damage and CRP we performed an area under the curve (AUC) analyses. Per patient, the CRP_{AUC} was defined as the sum of the 30 estimated CRP values, resulting from a piecewise linear mixed model which uses a linear time component for day 1-10 and a cubic time component for day 11-30. The conditioning regimen and the interactions between the particular regimen and time were also part of this model that also accounted for within person correlations by virtue of a random intercept. Likewise, the citrulline_{AUC} per patient was defined as the sum of the 30 estimated 10/citrulline values. We used the citrulline levels estimated by the linear mixed model described above and transformed these values into 10 times the inverse of the estimated value.

Comparisons between the impact of the different conditioning regimens on neutropenia, CRP_{AUC} and citrulline_{AUC} were studied using the Kruskal-Wallis test. The correlation between the degree of neutropenia and CRP_{AUC} versus citrulline_{AUC} and CRP_{AUC} was studied by Pearson correlation over the different regimens.

Comparison of the mean onset of acute GvHD between the different regimens was done using one-way ANOVA. Comparison of the incidence of ALI in relation to oral viridans streptococci (OVS) between the different regimens was done using the χ²-test.

Analyses were performed using SAS 8.2 and a P-value of <0.05 was considered to indicate statistical significance.

**RESULTS**

**STUDY POPULATION AND PATIENT CHARACTERISTICS**

Seventy-seven (77) patients received an autologous and 86 an allogeneic HSCT (Table 2). All but 14 patients received MA conditioning. Autologous HSCT was performed for patients with multiple myeloma (MM) and non-Hodgkin lymphoma, but allogeneic HSCT was employed for a greater variety of diagnoses including acute and chronic lymphatic and myeloid leukemia and myelodysplastic syndrome. NMA conditioning was employed to prepare patients who had received an autologous HSCT 4-6 months earlier for MM.
Figure 1. Course of citrulline and CRP in time after start of conditioning

Five MA and one NMA conditioning regimens are shown; A = HDM, B = BEAM, C = Ida-Cyclo-TBI, D = Cyclo-ATG-TBI, E = Cyclo-TBI, F = Cyclo-Flu. Observed values (•), mean values (○).
INTESTINAL DAMAGE
MA conditioning was associated with severe and prolonged intestinal damage shown by a rapid decline in citrulline to < 10 µmol/L, a mean of 10 days after starting chemotherapy. The mean nadir of citrulline was 4.5-7.0 µmol/L, and hypocitrullinemia lasted for more than one week in most patients (Figure 1A-E, 2A, Table 2). Hypocitrullinemia was most pronounced in patients receiving idarubicin in their conditioning, lasting approximately 18 days. In contrast, an early and short drop of citrulline level was noticed for NMA conditioning, but hypocitrullinemia was not evident for most patients (Figure 1F, 2A, Table 2).

Figure 2. Summary of the time course of citrulline (A) and CRP (B) for all 6 regimens

Day 1 is the day of start of conditioning. To correct for unobserved citrulline and CRP values we modeled the course of citrulline and CRP as described in methods.

1 = HDM, 2 = BEAM, 3 = Ida-Cyclo-TBI, 4 = Cyclo-ATG-TBI, 5 = Cyclo-TBI, 6 = Cyclo-Flu. Mean CRP in mg/L, mean citrulline in µmol/L.
INFLAMMATORY RESPONSE MEASURED BY C-REACTIVE PROTEIN AND FEVER

The course of CRP during HSCT of the different conditioning regimens is illustrated in Figure 1 and 2B. Within each type of MA conditioning, patients showed similar patterns of inflammatory response, although there was some variation in the precise onset, peak and resolution of CRP levels. Those without bacteraemia did not have a different course when compared to those with; although in general CRP levels were lower (data not shown). As for intestinal damage, the CRP response was highest in patients receiving idarubicin. Resolution of inflammation occurred with engraftment and restoration of the intestinal damage defined by rising citrulline levels.

In Cyclo-ATG-TBI conditioning the first peak of CRP was related to ATG induced lymphocyte depletion and cytokine release, but the second peak resembled that seen for the other MA regimens. Also some patients treated with Cyclo-TBI and BEAM had an early peak in CRP during conditioning, which was probably related to chemotherapy induced cytokine release.

Only a moderate inflammatory response occurred after NMA regimen (Figure 1F and 2B). Also, the timing was different when compared to MA regimens with a peak occurring early during conditioning and a second peak much later. The latter occurred during engraftment and thereby resembled to some extent the inflammatory complication designated engraftment syndrome.28

Virtually every patient who had received MA treatment developed fever as did 80% of those given NMA conditioning. Some patients receiving Cyclo-ATG-TBI and Cyclo-Flu also experienced an early episode with fever during conditioning (25 and 4 patients, respectively). In MA regimens fever occurred on days 12-14, 2-3 days after CRP had become elevated (Figure 1, Table 2). By contrast, fever occurred late during engraftment at a mean of day 16 after starting NMA conditioning.

RELATION INTESTINAL DAMAGE TO INFLAMMATION

In MA conditioning CRP levels started to increase from 10-11 days following the start of conditioning which coincided with the development of hypocitrullinemia (Figure 1A-E and 2). The peak of the inflammatory response coincided with the nadir of citrulline levels. Although interindividual differences existed the occurrence of inflammation was related to the development of intestinal damage in almost every patient.

Additional AUC analysis was used to grade the impact of conditioning on intestinal damage and CRP. There were significant differences in both CRP_{AUC} and citrulline_{AUC} between the various conditioning regimens (Kruskal-Wallis P <0.001), except between BEAM, Cyclo-TBI, and Cyclo-ATG-TBI. Interestingly, a very strong correlation between the degree of intestinal damage and the inflammatory response was seen for the different regimens (Pearson correlation 0.96, Figure 3). By contrast, there was only a weak correlation between the duration of neutropenia and inflammation (Pearson correlation 0.48, Figure 3).
Figure 3. Pearson correlation between mean degrees of neutropenia (NP in days) and inflammation ($CRP_{AUC}$) vs intestinal MBI ($Citrulline_{AUC}$) and inflammation over different regimens

1 = HDM, 2 = BEAM, 3 = Ida-Cyclo-TBI, 4 = Cyclo-ATG-TBI, 5 = Cyclo-TBI, 6 = Cyclo-Flu.

In univariate linear mixed model analysis, 10/citrulline, the type of conditioning regimen, neutropenia and bacteraemia were significantly associated with $\log CRP$. In multivariable analyses only citrulline and type of conditioning regimen remained important.

**STEM CELL TRANSPLANTATION COMPLICATIONS**

**BACTERAEMIA**

There was a significant difference between MA and NMA regimens regarding bacteraemia on the day of fever (Table 3) with up to 85% of patients experiencing bacteraemia predominantly
due to oral viridans streptococci (OVS) and CoNS after MA conditioning, compared with none of those receiving NMA ($P < 0.001$). OVS was recovered with CoNS in 20/55 (36%) of cases. A minority of patients experiencing a bacteraemia with CoNS on the day of fever had any clinical or radiological signs of a CVC related tunnel infection or thrombophlebitis at the same time (5/55 (9%)).

**ACUTE LUNG INJURY (ALI)**
The overall incidence of ALI was 18/163 (11%), with 14/18 (78%), being associated with OVS bacteraemia. Conversely, ALI affected 14/53 (26.4%) patients with OVS bacteraemia. However, this incidence varied significantly between conditioning regimens and was related to the severity of intestinal damage as ALI occurred in 3/22 (13.6%), 4/13 (30.8%) and 4/7 (57.1%) in patients receiving HDM, Cyclo-ATG-TBI, and Ida-Cyclo-TBI conditioning, respectively ($P = 0.03$) (Table 3).

**ACUTE GRAFT-VERSUS-HOST DISEASE**
No differences were seen in the total incidence of acute GvHD, although there were no cases of acute GvHD III-IV in the group with NMA; with only skin acute GvHD being encountered. However, there was a significant difference in the onset of acute GvHD. In Ida-Cyclo-TBI and Cyclo-TBI, despite receiving a partially T-cell-depleted graft, acute GvHD occurred early with a mean onset on day +25 post-HSCT. In both Cyclo-ATG-TBI and Cyclo-Flu the onset was delayed, with a mean onset on day +46 and +40 post-HSCT, respectively ($P = 0.02$).

**EARLY MORTALITY**
Overall, early mortality was low 6/163 (3.7%), and related to ALI and acute GvHD, and all but one death occurred following MA conditioning for an allogeneic HSCT.
Table 3. Hematopoietic stem cell transplantation complications

<table>
<thead>
<tr>
<th>Post-HSCT complications</th>
<th>HDM (N=56)</th>
<th>BEAM (N=21)</th>
<th>Ida-Cyclo-TBI (N=28)</th>
<th>Cyclo-ATG-TBI (N=34)</th>
<th>Cyclo-TBI (N=10)</th>
<th>Cyclo-Flu (N=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteraemia on day of fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVS</td>
<td>29 (39.3%)</td>
<td>10 (62.5%)</td>
<td>18 (64.3%)</td>
<td>29 (38%)</td>
<td>6 (60%)</td>
<td>0</td>
</tr>
<tr>
<td>CoNS</td>
<td>12 (21.4%)</td>
<td>6 (30%)</td>
<td>7 (25%)</td>
<td>13 (38%)</td>
<td>21 (65%)</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Concomitant OVS/CoNS</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Candidemia</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clinically defined infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phlebitis superficial vein</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tunnel infection/infected thrombosis</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Probable/Proven IA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALI</td>
<td>5/56 (7.1%)</td>
<td>2/21 (9.5%)</td>
<td>6/28 (21.5%)</td>
<td>4/34 (11.8%)</td>
<td>2/10 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>ALI following OVS bact.</td>
<td>3/22 (13.6%)</td>
<td>1/6 (16.7%)</td>
<td>4/7 (57.1%)</td>
<td>4/13 (30.8%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Early mortality (day + 30)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ALI Acute GvHD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>aGvHD I-IV, all (N, %)</td>
<td>NA</td>
<td>NA</td>
<td>13/28 (46%)</td>
<td>12/34 (28%)</td>
<td>6/10 (60%)</td>
<td>6/14 (43%)</td>
</tr>
<tr>
<td>Grade II-IV</td>
<td>-</td>
<td>-</td>
<td>8 (28.5%)</td>
<td>7 (20.5%)</td>
<td>3 (30%)</td>
<td>4 (28.5%)</td>
</tr>
<tr>
<td>Grade III-IV</td>
<td>NA</td>
<td>NA</td>
<td>2 (7%)</td>
<td>2 (6%)</td>
<td>1 (10%)</td>
<td>-</td>
</tr>
<tr>
<td>Onset from day HSCT, mean (95%CI)</td>
<td>NA</td>
<td>NA</td>
<td>26 (16-36)</td>
<td>46 (29-63)</td>
<td>23 (19-27)</td>
<td>40 (19-61)</td>
</tr>
</tbody>
</table>

IA = invasive Aspergillosis, ALI = acute lung injury, OVS bact. = bacteraemia with oral viridans streptococci. Grading of acute GvHD was done according to the criteria of Przepiorka et al27 and probable/proven IA was defined according to EORTC/MSG consensus definitions.25

**DISCUSSION**

In this study we show the course and extent of intestinal damage and inflammatory responses following various conditioning regimens used to prepare for a HSCT. There was a striking pattern of inflammatory response coinciding with the occurrence of severe intestinal damage for patients receiving MA conditioning, defined by hypocitrullinemia.20 Moreover, the degree of intestinal damage seemed the most important determinant of inflammation and was highly correlated with the magnitude of the inflammatory response measured by CRP. Neither neutropenia nor bacteraemia had a major impact on this. The close relationship between intestinal damage and inflammation was further underscored by the fact that the NMA regimen resulted in only a modest inflammatory response with a completely different time course, and the virtual absence of severe intestinal damage. Consequently, intestinal damage appears the primary determinant
of inflammation following MA conditioning with chemo- and radiotherapy in the setting of autologous and allogeneic HSCT.

While there are limitations associated with retrospective analysis and the potential for bias resulting from selection, the relationship of intestinal damage to HSCT complications was remarkable. As expected, there was a significant difference in occurrence of bacteraemia between those receiving MA and NMA conditioning. The similar duration of neutropenia and the marked difference in intestinal damage, suggest that the gut may have been the origin of bacteraemia. Moreover, most pathogens recovered from blood cultures are known residents of the gut. Notably, a considerable proportion of patients had bacteraemia with both CoNS and OVS, which was probably due to simultaneous intestinal translocation.

A strong relationship has been found between the occurrence of OVS bacteraemia and septic shock and ALI in neutropenic patients, which was explained by virulence factors and pulmonary cytotoxicity of chemotherapy. Interestingly, we saw that in patients with OVS bacteraemia the incidence of ALI was related to the degree of intestinal damage. It is known that barrier dysfunction facilitates bacterial translocation, but intestinal damage also seems to determine the resulting inflammatory response. This was confirmed in our linear mixed model, which showed citrulline but not bacteraemia related to the CRP response. Although ALI seems directly associated with OVS bacteraemia this might be only coincidental, as both complications are consequences of severe intestinal damage. So intestinal damage ‘primes’ the immune system with subsequent aggravated cytokine release following activation of pattern recognition receptors from translocating microbial motifs.

This ‘priming’ of the immune system also applies to the occurrence acute GvHD in which the role of intestinal damage has been acknowledged. Although we found no apparent differences in the citrulline and CRP levels between patients with and without acute GvHD within any given regimen, between regimens there was a clear difference in the kinetics of acute GvHD. In addition severe acute GvHD did not develop after NMA, as opposed to 6-10% after MA, and GvHD of liver or gut did not occur. The early onset of acute GvHD after Ida-Cyclo-TBI and Cyclo-TBI suggests that the tissue inflammation resulting from the profound intestinal damage, contributes to the accelerated allo-reactive T-cell response, even in the setting of partial T cell-depletion. The delay in onset of acute GvHD in patients conditioned with ATG results from additional in vivo T cell-depletion creating a ‘time-window’ between the intestinal damage induced inflammation and T cell recovery. After NMA treatment we also saw a delay in the onset of acute GvHD, which is in accordance with previous data from studies in mice and humans. This altered kinetics of acute GvHD was, at least in part, related to the absence of significant intestinal damage and tissue inflammation. Differences in the kinetics of acute GvHD in NMA regimens have been largely attributed to alterations in antigen presenting cell chimerism, T cell chimerism and regulatory T cell activation, but our data underscore the role conditioning-induced intestinal damage plays in the complex pathogenesis of acute GvHD. Several studies have shown correlations between CRP and the occurrence of HSCT complications.
but they all used different cut-off values.\textsuperscript{5, 7} CRP is not a specific marker since chemotherapy and ATG and, the process of engraftment itself, elicit inflammatory responses. Hence it is not possible to identify who is at risk or when that risk might occur. Citrulline could provide an alternative, because it is a specific marker of enterocyte mass, which decreases only when there is intestinal damage. Furthermore, citrulline levels correspond with the inflammatory responses following MA conditioning, and more importantly with HSCT complications. Clearly it is necessary to confirm the predictive value of citrulline for individual patients and to define cut-off values more precisely. Classifying other conditioning regimens, by means of measuring citrulline can already help determine the need for antimicrobial prophylaxis, hospital care, and the use of anti-inflammatory treatment.

Given the role of intestinal MBI in complications after HSCT studies should explore ways to ameliorate cytotoxic therapy-induced intestinal damage in order to reduce inflammatory complications associated with myeloablative conditioning therapy.

REFERENCES


5. Schots R, Van Riet I, Othman TB et al. An early increase in serum levels of C-reactive protein is an independent risk factor for the occurrence of major complications and 100-day transplant-related mortality after allogeneic bone marrow transplantation. Bone Marrow Transplant 2002; 30: 441-446.


CHAPTER 8

MUCOSITIS NOT NEUTROPENIA DETERMINES BACTERAEMIA AMONG HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

ABSTRACT

INTRODUCTION
In the 1960s it was reported that infectious complications were the main cause of fever during neutropenia that followed cytotoxic treatment. More recently, mucositis, also called mucosal barrier injury (MBI) has become recognised as an important determinant of the inflammatory response and infectious complications in hematopoietic stem cell transplant (HSCT) recipients.

PATIENTS AND METHODS
The objective of this prospective study was to determine the impact of intestinal MBI, as measured by plasma citrulline, and neutropenia on the systemic inflammatory response (C-reactive protein) and the occurrence of bacteraemia among two cohorts of HSCT recipients one composing 18 patients who had been treated with a myeloablative (MA) regimen (High dose melphalan: HDM) and the other involving 19 patients who had received the non-myeloablative (NMA) regimen (Cyclophosphamide and Fludarabine: Cyclo-Flu). Blood cultures were obtained for surveillance from admission onwards as well as at the onset of fever.

RESULTS
The MA regimen induced severe intestinal MBI manifest by citrullinaemia < 10 μmol/L which was accompanied by an inflammatory response and bacteraemia affected 8 (44%) of 18 patients and coincided with the nadir of citrullinaemia. By contrast, those who had been treated with the NMA regimen did not develop severe intestinal MBI, had a moderate inflammatory response and only 2 (11%) of the 19 patients developed bacteraemia. However, both groups experienced profound neutropenia and its duration was significantly longer for those receiving the NMA regimen.

CONCLUSION
This study suggests that severe intestinal MBI i.e. citrullinaemia < 10 μmol/L defines the period of risk of bacteraemia better than does neutropenia and that measuring plasma citrulline may prove useful in deciding who needs empirical antimicrobial therapy and when.
INTRODUCTION

Conditioning therapy is given to prepare for a hematopoietic stem cell transplant (HSCT) and can be myeloablative or not (reduced intensity).1 Myeloablation is typically achieved using high doses of chemotherapy with or without radiotherapy and renders the recipient vulnerable to infectious complications which can result in significant morbidity and mortality despite markedly improved supportive care.

In the 1960s it became apparent that fever was often the only sign of an infection during neutropenia.2 This phenomenon is now referred to as “febrile neutropenia” and has become recognized as one of the most important complications of chemotherapy. So much that prevention and treatment with antimicrobial drugs has become an established standard of care often augmented by the use of growth factors e.g. G-CSF.3 However, a substantial number of patients remain febrile without an infection ever being documented.4 This is not so surprising since fever is induced by cytokines, the release of which is by no means solely related to infections. Consequently some patients are over-treated with antibiotics increasing the risk of adverse effects as well as antibiotic resistance among bacteria.

Myeloablative conditioning regimens are designed to ablate the bone marrow but they also cause severe damage to the mucosal barrier which manifests itself as mucositis of mouth and the gastrointestinal (GI) tract particularly the small intestine.5,6 The amino acid citrulline is produced by enterocytes and released into the bloodstream and has been shown to be a biomarker for intestinal mucosal barrier injury (MBI).7,8 It has also been shown that HSCT recipients receiving chemotherapy experienced more fever (with or without infections) in the presence of severe MBI9 and that intestinal MBI plays a role in the occurrence of bacteraemia.10,11

The aim of the current prospective study was to determine the impact of intestinal MBI (as determined by citrulline) and neutropenia on the systemic inflammatory response (as determined by C-reactive protein) and the occurrence of bacteraemia (daily blood cultures) among patients receiving either a myeloablative (MA) (high dose melphalan: HDM) or a non-myeloablative (NMA) regimen (Cyclophosphamide and Fludarabine: Cyclo-Flu) before HSCT.1

METHODS

PATIENT MANAGEMENT

From January 2007 to December 2008, 37 patients participated in this observational single centre study. A cohort of 18 patients received an autologous stem cell transplant (8 female and 10 male) after preparation by the MA regimen. Another cohort of 19 patients (9 female and 10 male) were given an allogeneic stem cell transplant following the NMA regimen.

All patients had given their informed consent to the prospective collection of plasma samples for investigational use. Formal approval of this study was not required by the local ethics
committee (CMO regio Arnhem-Nijmegen) provided that patient data were handled anonymously in compliance with Dutch laws on privacy.

Patients’ characteristics are shown in Table 1A.

<table>
<thead>
<tr>
<th>Table 1A. General characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conditioning</strong></td>
</tr>
<tr>
<td>Age in years: mean (range)</td>
</tr>
<tr>
<td>Gender: male/female</td>
</tr>
<tr>
<td>Diagnoses</td>
</tr>
<tr>
<td>Multiple myeloma/plasma cell leukemia (N; %)</td>
</tr>
<tr>
<td>B-cell Non Hodgkin’s lymphoma (N; %)</td>
</tr>
<tr>
<td>MDS/AML (N; %)</td>
</tr>
<tr>
<td>B-cell chronic lymphocytic leukemia (N; %)</td>
</tr>
<tr>
<td>Type of HSCT</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

HDM was given intravenously (iv.) at a dose of 100 mg/m² for two consecutive days (day 1 and 2), followed by an autologous transplant on day 4. All recipients of this regimen were managed with a triple-lumen central venous catheter (CVC). The patients who received the NMA regimen were given Cyclophosphamide (Cyclo) at a dose of 1200 mg/m² and Fludarabine (Flu) at a dose of 30 mg/m², both given i.v. for 4 consecutive days (day 1 to 4), followed by an allogeneic stem cell transplant from a matched related donor on day 7. Only one of these patients had a CVC.

Antimicrobial prophylaxis consisted only of ciprofloxacin and valaciclovir which were given from admission onwards. Fluconazole was only given to patients who were shown to be colonized by *Candida albicans*. Ceftazidime was given empirically for fever that occurred during neutropenia. No hematopoietic growth factors or keratinocyte growth factors were used.

The course of neutropenia, axillary temperature, citrulline and C-reactive protein levels and the occurrence of bacteraemia were monitored.

**NEUTROPHIL COUNT**

The absolute neutrophil count (ANC) was determined daily and neutropenia was defined as an ANC ≤ 0.5 x 10⁹/L. The duration and the depth of neutropenia was recorded for each patient.

**CITRULLINE ANALYSIS**

The protocol required plasma to be obtained daily for citrulline assay but, in practice, this was
not always done though it was collected at least three times a week. Plasma was prepared and stored at –80°C for later analysis. Citrulline concentrations were measured by a standard procedure for determining amino acids using high-performance liquid chromatography (Shumadzu© Kyoto, Japan) and levels below 10 μmol/L were designated hypocitrullinaemia and considered to represent severe intestinal damage.7

TEMPERATURE
The axillary temperature was measured 4-5 times a day and fever was defined by a single temperature of ≥ 38.5 °C. The maximum daily temperature was used for analysis.

C-REACTIVE PROTEIN (CRP)
CRP levels were determined every day until discharge using turbidimetric immunoassay (Aeroset©, Abbott). The day of maximum CRP and the highest level of CRP (CRP max) were recorded.

INFECTIOUS COMPLICATIONS, BLOOD CULTURES AND BACTERAEMIA
Infections were defined according to the Consensus definitions of Immunocompromised Host Society.15 The study protocol recommended blood cultures be obtained daily but, in practice, they were taken at least three times a week in every case. When a central venous catheter (CVC) was present, 10 mL of blood from each lumen was drawn into an aerobic blood culture bottle. When there was no CVC, 10 mL of peripheral blood was drawn into an aerobic culture bottle.

At the onset of fever, an extra of 40 mL of blood was obtained for culture from a peripheral vein together with 10 ml from each lumen of the CVC when present (same protocol as described earlier).10

Bacteraemia was defined by a single blood culture yielding any bacterial species except for coagulase-negative staphylococci (CoNS) which required the same isolate to be recovered from two separate blood cultures.13

DATA-ANALYSIS
We used descriptive statistics for each time point from the day of starting conditioning regime. Continuous variables are expressed as mean values together with a 95% confidence interval (CI). The categorical variables are displayed as absolute number and percentage. Results from different regimens in the same patient were considered statistically independent. As citrulline was not measured daily, the real nadir might occur between two measurements and hence could be missed. To overcome this problem and to be able to estimate the length of time citrulline levels were < 10 μmol/L, we transformed the values to their logarithms and modelled the course of citrulline as a function of time using a linear mixed model treating the ‘patient’ as a random factor, and the interaction ‘treatment’ by ‘day after start of conditioning’
as fixed factors. This modelling approach allowed us to deal adequately with missing values. The t-test or Wilcoxon test was used to compare the two treatment regimens with respect to continuous variables. Fisher’s exact test was used to compare the two regimens with respect to frequencies. To estimate the 95% CI for the median a distribution free method was used. A P-value of < 0.05 was considered to indicate significance. SAS version 9.2 software (SAS, Inc. Cary, NC, USA) was used for statistical analysis.

RESULTS

THE COURSE OF NEUTROPENIA
Patients in both groups developed profound neutropenia which was significantly longer (3 days) among those who had received the NMA regimen than for the MA regimen (see Table 1B; Figure 1).

<table>
<thead>
<tr>
<th>Conditioning</th>
<th>HDM N=18</th>
<th>Cyclo-Flu N=19</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of neutropenia in days, mean (95%CI)</td>
<td>11 (8-13)</td>
<td>14 (12-15)</td>
<td>0.0048</td>
</tr>
<tr>
<td>Citrulline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of citrulline &lt;10 µmol/L in days, mean (95%CI)</td>
<td>10 (5-12)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Citrulline nadir (µmol/L): mean (95%CI)</td>
<td>5.6 (5.0-6.2)</td>
<td>16.6 (15.1-18.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRPmax (mg/L): mean (95%CI)</td>
<td>119 (99-156)</td>
<td>60 (38-94)</td>
<td>0.01</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever, N (%)</td>
<td>16 (89%)</td>
<td>13 (68%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fever, day of start conditioning, mean (95%CI)</td>
<td>12.2 (11.1-13)</td>
<td>14.4 (13.2-15.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteraemia, N (%)</td>
<td>8 (44%)</td>
<td>2 (11%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oral viridans staphylococci, N</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci, N</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Other: Escherichia coli, N</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

THE COURSE OF CITRULLINE
The patients who had received the MA regimen developed hypocitrullinaemia with a rapid decline of citrulline levels to <10 µmol/L a mean of 10 days after starting chemotherapy. The mean nadir of citrulline was 5.6 (95% CI: 5.0-6.2) µmol/L and hypocitrullinaemia lasted for 10 (95% CI: 5-12) days.
By contrast, no hypocitrullinaemia was evident among those who had received the NMA regimen and no real decline in citrulline was seen. The mean nadir of citrulline for this group was 16.6 (95% CI: 15.1-18.3) µmol/L (figure 1).

**Figure 1.** Total days of hypocitrullinaemia and neutropenia for each patient either treated with the myeloablative regimen (HDM) or non-myeloablative regimen (Cyclo-Flu)

(Thick grey horizontal line indicates the mean and thinner grey vertical line the 95% CI). The MA regimen induced severe intestinal MBI manifest by citrullinaemia < 10 µmol/L, whereas no severe intestinal MBI was observed in those treated with the NMA regimen. Both groups experienced profound neutropenia and its duration was significantly longer for those receiving the NMA regimen (P=0.0048).

**INFLAMMATORY RESPONSE MEASURED BY CRP AND FEVER**

Significantly higher CRP values were seen for the cohort who had been given the MA regimen with a mean CRPmax of 119 mg/L versus 60 mg/L for the cohort who had received the NMA regimen. The course of CRP also reflected the course of citrulline, whereas only a moderate inflammatory response was seen among those treated with the NMA regimen.

Most patients developed fever, which affected 16 (89%) of the patients given the MA regimen and 13 (68%) of those given the NMA regimen. The mean duration of fever was 2.5 days for
both groups. In the MA group fever occurred two days after the occurrence of hypocitrullinaemia approximately 12 days after starting chemotherapy, compared with approximately 15 days for those given the NMA regimen. The two patients in the NMA group who had the longest duration of fever during neutropenia, respectively 7 and 8 days, had infiltrative changes on their chest X-ray; one of which was associated with probable aspergillosis. The length of neutropenia until fever occurred was also significantly longer in the NMA regimen (respectively a mean of 5 days in the MA group versus 11 days in the NMA group \( P=0.02 \)).

**BACTERAEMIA**

*Figure 2.* Relationship for patients receiving HDM between citrulline, neutropenia, bacteraemia and the day after starting conditioning

All patients who developed bacteraemia (inner figure) had hypocitrullinaemia at that moment. Positive blood cultures are shown as percentage of all blood cultures taken that day. Four patients had repeated positive blood cultures - respectively 3 patients had positive blood cultures on two consecutive days (2 with viridans streptococci, 1 with coagulase-negative staphylococci) and 1 patient had positive blood cultures with coagulase-negative staphylococci on three consecutive days.

Bacteraemia was experienced by 8 (44%) of those in the MA group (5 cases due to oral viridans streptococci (OVS), and 3 cases due to CoNS) compared with 2 (11%) in the NMA treated group \( P<0.001 \). None of the patients experiencing a bacteraemia with coagulase negative staphylococci had any clinical or radiological signs of a CVC related tunnel infection or thrombophlebitis.
Moreover, all patients treated with the MA regimen who experienced bacteraemia had hypocitrullinaemia (Figure 2) although the course and duration of hypocitrullinaemia was similar whether or not bacteraemia had occurred. Bacteraemia developed a day before fever in two cases. In six cases it was detected in blood cultures obtained on the same day fever developed. One of the patients who had received the NMA regimen had bacteraemia due to *Escherichia coli* that was resistant to ciprofloxacin, but responded well to ceftazidime. The other patient developed bacteraemia due to coagulase negative staphylococci which was associated with thrombophlebitis in his right arm. Both patients developed fever on the same day that bacteraemia was discovered.

**DISCUSSION**

In this study, the MA regimen HDM induced severe intestinal MBI which was an important determinant of the inflammatory response and bacteraemia. All episodes of bacteraemia in the MA treated group were due to either CoNS or OVS most likely as a direct consequence of our use of ciprofloxacin, which prevents bacteraemia due to enteric Gram-negative bacilli but provides a selective advantage to these Gram positive bacteria as they are less susceptible to the quinolone. These bacteria almost certainly originated from the gastrointestinal tract as has been shown by molecular analysis and clinical observations.10,17-20 The patients who had been treated with the NMA regimen did not develop severe intestinal MBI, had only a moderate inflammatory response and experienced significantly fewer bacteraemic episodes even though they experienced profound neutropenia for a significantly longer duration than that resulting from the MA regimen. Also, the time from the start of neutropenia to the development of fever was significantly longer being around 5 days.

These results indicate that hypocitrullinaemia identifies severe intestinal MBI induced by MA regimens and better defines the risk period for developing an inflammatory response as well as bacteraemia than does neutropenia. This might also explain why giving antibiotics pre-emptively to reduce the occurrence of fever usually fails, as there will be little or no impact on MBI.21 Rather, we should reconsider our current approach towards infectious complications among HSCT recipients. Typically, antimicrobial prophylaxis is given for as long as the patient remains neutropenic. Empirical antibiotic therapy is then started promptly once fever develops and after blood cultures have been obtained. In our study, bacteraemia could be detected even before fever occurred and may not have been registered at all later on. Moreover, fever is often an expression of inflammation that is unrelated to the occurrence of bacteraemia or any other manifest infection.

Antimicrobial consumption could also be reduced by half if only those with the severe mucositis are treated. Citrulline could be useful in this regard22 to explore whether knowing the levels might indeed help to determine which patient requires antimicrobial prophylaxis, hospital admission or empirical antimicrobial therapy.
Furthermore the results of our study suggest patients given an NMA regimen may not require antimicrobial prophylaxis provided empirical antibiotic therapy is started promptly at the onset of fever. Therapy could then be stopped after 2-3 days if there is no evidence of bacteraemia or other infectious complications. These differentiated approaches would also help to lower the risk for side effects to antimicrobial agents, and help reduce emergent resistance and health care costs. However to confirm our observations a large prospective trial is needed to investigate this more thoroughly before a change in our common approach can be recommended. In conclusion, our results show that the period of severe intestinal MBI following treatment with a MA regimen is manifest by hypocitrullinaemia and that this, rather than neutropenia, defines the period of risk of bacteraemia. A more tailored approach to HSCT related infectious complications that also focuses on the occurrence of intestinal MBI would benefit patients in particular and health care in general not least by reducing costs and lowering the risk of antimicrobial resistance.

REFERENCES


PART IV

CONSEQUENCES OF USING CITRULLINE IN CLINICAL PRACTICE
CHAPTER 9

IMPACT OF PALIFERMIN ON INTESTINAL MUCOSITIS OF HSCT RECIPIENTS AFTER BEAM

AHE Herbers, WJFM van der Velden, AFJ de Haan, JP Donnelly and NMA Blijlevens.
Bone Marrow Transplant 2014; 49(1): 8-10.
ABSTRACT

INTRODUCTION
Mucosal barrier injury (MBI) plays an important role in the inflammatory and infectious complications seen in HSCT recipients after intensive chemotherapy and radiotherapy. Palifermin, a recombinant human keratinocyte growth factor, is registered for reduction of oral MBI. Less is known about intestinal MBI, mainly due to the inaccessibility of the gut. Recently, the aminoacid citrulline has been shown to be an useful marker of intestinal MBI.

In the present single centre matched-control study, we examined the efficacy of palifermin for the prevention and reduction of MBI induced by cytotoxic therapy and its impact on the inflammatory response and the occurrence of fever and bacteraemia.

PATIENTS AND METHODS
Between January 2007 and November 2008, 23 autologous HSCT patients conditioned with BEAM were enrolled to receive palifermin and compared to matched historic controls.

RESULTS
Palifermin reduced oral MBI but had no relevant impact on intestinal MBI. 16 of the 21 (76%) patients that did not receive palifermin developed fever during neutropenia compared to 13 (57%) patients of those who were treated with palifermin. There was no impact on the occurrence of bacteraemia. Lower levels of CRP were seen in the palifermin group. This difference in CRP course was attributable to those without bacteraemia.

CONCLUSION
The matched-control study failed to show a clinical relevant impact of palifermin on intestinal MBI, though there was a reduced inflammatory response and less fever during neutropenia among patients who had no bacteraemia.
INTRODUCTION

Mucositis is the clinical manifestation of mucosal barrier injury (MBI) which plays an important role in the inflammatory and infectious complications seen during the leucopenic phase following hematopoietic stem cell transplantation (HSCT).1 Palifermin is a recombinant human keratinocyte growth factor found to reduce oral MBI after TBI-containing regimens to prepare for autologous HSCT but not in other regimens.2-4 Much less is known about palifermin’s impact on intestinal MBI, mainly due to difficulties in examining the gut. Johannson et al. suggested a protective effect of palifermin measured by permeability tests on the intestinal barrier in BEAM treated HSCT recipients.5 The amino acid citrulline appears more useful as marker of intestinal MBI than do permeability tests.6 So the aim of this single-centre matched-control study was to explore the efficacy of palifermin in reducing intestinal MBI, measured by plasma citrulline after BEAM therapy. Other objectives were to evaluate the effect, if any, of palifermin on the inflammatory response using C-reactive protein (CRP) as a marker, on the incidence of fever during neutropenia and the occurrence of bacteraemia.

PATIENTS AND METHODS

Between January 2007 and November 2008, a cohort of 23 patients suffering from malignant lymphoma underwent an autologous HSCT after BEAM (carmustine (300mg/m² day 1 (HSCT-day -6), etoposide (200mg/m²/day from day 2 till 5 (HSCT day: -5 till -2)), cytarabine (200mg/m²/day from 2 till day 5 (HSCT day: -5 till -2)) and melphalan (140 mg/m² day 6 (HSCT day -1)) and was treated with palifermin. Palifermin was administered once daily at the standard dose of 60 μg/kg/day intravenously for 3 days before starting BEAM (day -2 till 0 (HSCT day: -9 until -7) and again for 3 days after autologous HSCT (day 7 till 9 after start of BEAM or HSCT day: 0 until +2).2 The matched-controlled cohort consisted of patients with malignant lymphoma and were all transplanted after BEAM from July 2004 to December 2006. None of these patients received glutamine or any other agent with a potential protective influence on MBI. Every patient received a stem cell graft with at least 2.0 x 10⁶ CD34+ cells/kg without colony stimulating growth factors and remained in hospital until neutrophil recovery. All were managed with a central venous catheter (CVC) and antimicrobial prophylaxis consisted of ciprofloxacin and valaciclovir. At the onset of fever (defined by a single axillary temperature of ≥ 38.5 °C) during neutropenia (absolute neutrophil count ≤ 0.5 x 10⁹/L) 40 mL of peripheral blood was obtained for culture together with 10 mL from each lumen of the CVC, appropriate specimens were obtained from any site of suspected local infection including a chest radiograph, and empirical therapy with ceftazidime was started.7 The following were done prospectively from the day before chemotherapy was started (day 0) until discharge: absolute neutrophil count,
CRP levels, citrulline levels, oral mucositis scores (daily mucositis score (DMS), WHO toxicity score, daily gastrointestinal mucositis score (DGS) and any event according to the CTC-AE v3.0 including fever, bacteraemia, and adverse drug reactions.\(^8\)

**RESULTS AND DISCUSSION**

Patient characteristics were well balanced between the two groups (Table 1a).

<table>
<thead>
<tr>
<th>Table 1A. General characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Palifermin</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Total of patients (N)</td>
</tr>
<tr>
<td>Age in years: mean (range)</td>
</tr>
<tr>
<td>Gender: male/female</td>
</tr>
<tr>
<td>Diagnoses</td>
</tr>
<tr>
<td>B-cell Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>T-cell Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Type of HSCT</td>
</tr>
</tbody>
</table>

The administration of palifermin was generally safe though every patient experienced a degree of skin toxicity typically a rash scored as CTC grade 1 for 11 patients and grade 2 for the remaining 12 patients. None of the patients developed pancreatitis or arthralgia.

Although there was a statistically significant difference in the peak DGS and nadir of citrulline levels palifermin had no discernible clinical effect on intestinal MBI as all patients developed severe enterocyte damage reflected by the presence of hypocitrullinaemia (i.e. citrulline below 10 \(\mu\)mol/L) and the DGS was in the range that corresponds to mild toxicity in both study groups. Nevertheless, the duration of hypocitrullinaemia was 2 days shorter and the nadir was less pronounced (Table 1b, 1c) among patients treated with palifermin.
Table 1B. General outcome measure of neutrophils, citrulline, inflammation and bacteraemia

<table>
<thead>
<tr>
<th>Number of patients (%)</th>
<th>Palifermin N=23 (100%)</th>
<th>Control N=21 (100%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration neutrophils ≤ 0.5 x 10⁹/L, days, mean (95%CI)</td>
<td>10 (8-10)</td>
<td>9 (8-10)</td>
<td>ns</td>
</tr>
<tr>
<td>Citrulline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day of citrulline &lt; 10 µmol/L, mean (95%CI)</td>
<td>9.2 (8.1-10.3)</td>
<td>8.0 (7.8-8.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Duration Citrulline &lt; 10 µmol/L, days, mean (95%CI)</td>
<td>10 (8-13)</td>
<td>12 (11-14)</td>
<td>0.02</td>
</tr>
<tr>
<td>Nadir (µmol/L): mean (95%CI)</td>
<td>5.2 (4.6-5.9)</td>
<td>4.1 (3.6-4.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Time of nadir, days from start BEAM, mean</td>
<td>14</td>
<td>15</td>
<td>ns</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRPmax (mg/L): mean (95%CI)</td>
<td>86 (68-108)</td>
<td>130 (100-169)</td>
<td>0.02</td>
</tr>
<tr>
<td>number of patients (%) with fever</td>
<td>13 (56.5%)</td>
<td>16 (76.2%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Time of onset fever, days from start BEAM, mean (95%CI)</td>
<td>12 (11.1-13)</td>
<td>11 (10.2-11.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>number of patients (%)</td>
<td>11 (47.8%)</td>
<td>10 (47.6%)</td>
<td>ns</td>
</tr>
<tr>
<td>Time of onset of bacteraemia in days from start BEAM, mean (95%CI)</td>
<td>13 (11-14)</td>
<td>12 (10-13)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table 1C. General outcome measure of oral mucositis and daily gut score

<table>
<thead>
<tr>
<th>Number of patients (%)</th>
<th>Palifermin N=17 (73.9%)</th>
<th>Control N=14 (66.7%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral mucositis scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSmax: mean (95%CI)</td>
<td>5.1 (4.3-5.8)</td>
<td>6.6 (5.7-7.4)</td>
<td>0.0071</td>
</tr>
<tr>
<td>number of patients (%) with DMS Grade II or higher</td>
<td>1 (5.9%)</td>
<td>3 (21.4%)</td>
<td>ns</td>
</tr>
<tr>
<td>WHOmax: mean (95%CI)</td>
<td>1.5 (1.3-1.8)</td>
<td>2.3 (2.1-2.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>number of patients (%) with WHO grade II or higher</td>
<td>8 (47.1%)</td>
<td>14 (100%)</td>
<td>0.0013</td>
</tr>
<tr>
<td>Daily gut score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DGSmax: mean (95%CI)</td>
<td>3.7 (3.5-3.9)</td>
<td>4.2 (3.7-4.7)</td>
<td>0.041</td>
</tr>
<tr>
<td>number of patients (%) with DGS Grade II or higher</td>
<td>0 (0%)</td>
<td>1 (7.1%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Significantly higher oral mucositis scores were seen in the control group, with the peak DMS being seen on day 10 and the peak WHO toxicity score being seen on day 12 in the control group, and later on day 13 and 14 respectively in the palifermin group, but there were no differences in the incidence.
Ten control patients and 11 palifermin patients developed bacteraemia, with 4 and 5 cases respectively being due to viridans streptococci and 6 cases in each group being due to coagulase-negative staphylococci without there being any clinical signs of a catheter-related infection. Although both the oral cavity and gut could be the origin of these bacteraemias our data favour the latter as we found that every patient developing bacteraemia had hypocitrullinaemia. Moreover, patients treated with palifermin had significantly less oral mucositis yet the rate of bacteraemia was not lower.9-12 Hence, the onset of hypocitrullinaemia appears to delineate the period of highest risk for bacteraemia irrespective of neutropenia, at least among autologous HSCT recipients.12 Lower levels of CRP were seen in the palifermin group and less febrile neutropenia (Table 1B). The difference in CRP course was attributable to those without bacteraemia (Figure 1). As mucositis is the main driver of inflammation after myeloablative therapy our results suggest that palifermin exerted some effect on the severity of oral and intestinal MBI leading to a reduction in inflammation.13 Furthermore, there are reports that palifermin mediates immuneregulation by shifting the balance between Th1-cell towards Th2, thereby down-regulating proinflammatory cytokines and up-regulating interleukin(IL)-4 and IL-13.14,15 Palifermin might also have a protective action on endothelium and fibroblasts.16,17

**Figure 1.** Course of mean CRP in patients without bacteraemia after the start of BEAM

In the patients without bacteraemia there is an overall significant palifermin*day interaction: P <0.0001. Between day 12 and 17: mean C-reactive protein (CRP) is significantly lower in palifermin-treated group (P <0.05).
CONCLUSION

This study failed to detect any clinically relevant impact of palifermin on intestinal MBI. However, the reduction in the inflammatory response and febrile neutropenia in patients who had no bacteraemia is intriguing and merits further investigation. Spielberger et al., also found a lower incidence of febrile neutropenia among patients treated with palifermin.\(^9\) If fever is, in fact, induced by the inflammatory response to cytotoxic induced therapy rather than infection per se it might have important consequences for clinical practice by reducing the consumption of antimicrobials given empirically to HSCT recipients for the treatment of persistent unexplained fever thereby lowering the risk of side-effects and the induction of microbial resistance.

REFERENCES

3. Blijlevens N, de Chateau M, Krivan G et al. In a high-dose melphalan setting, palifermin compared with placebo had no effect on oral mucositis or related patient’s burden. Bone Marrow Transplant 2013; 48(7): 966-971.


CHAPTER 10

CLEVER PLANNING OF HEMATOPOIETIC STEM CELL TRANSPLANTATION AVOIDS FEBRILE COMPLICATIONS AT THE WEEKEND

AHE Herbers, WJFM van der Velden, AFJ de Haan, JP Donnelly and NMA Blijlevens.
Submitted.
ABSTRACT

INTRODUCTION
Infection is a major cause of treatment-related morbidity and mortality of patients receiving hematopoietic stem cell transplantation (HSCT) to treat cancer. The immunosuppressive state that accompanies HSCT means that the signs and symptoms of an overt infection are often rather subtle. Therefore, optimal care can be delivered only by closely monitoring the patient. With less experienced physicians on call, fewer nurses on duty and limited time for consultation the care of a HSCT recipient during the weekends is seldom up to the usual standard of care and may actually expose the patient to decisions based more on a “gut feeling” rather than on objective criteria.

PATIENTS AND METHODS
To explore this notion we analyzed retrospectively a relatively uniform cohort of 24 patients undergoing a HSCT for multiple myeloma to determine whether the day of starting conditioning with high dose melphalan (HDM) might predict whether fever or bacteraemia would occur in the weekend.

RESULTS
Of the 24 patients, 18 (75%) developed fever and 12 (50%) developed bacteraemia. Patients who started HDM on a Tuesday had fever during the weekend following HSCT, and those who developed bacteraemia, also did so in the same weekend. By contrast, none of the patients who started HDM on a Friday, Saturday or Sunday developed fever during the weekend (p<0.001).

CONCLUSION
Better planning of conditioning therapy will reduce the chances that fever and bacteraemia will occur during the weekend. This would help limit unwarranted use of antibiotics and the unnecessary ordering of diagnostic tests hence reducing health care-related costs.
INTRODUCTION

Patients who receive a HSCT are vulnerable to infections because of intensive conditioning regimens that disintegrate their natural host defences.\textsuperscript{1} The resultant absence of granulocytes and mucosal barrier injury (MBI) allow resident micro-organisms ready access to the bloodstream. Under these circumstances, a relatively small number of bacteria can cause a serious disease, including sepsis and shock. The absence of granulocytes mutes the symptoms of an emerging infection with fever often being the only warning sign.\textsuperscript{2} Therefore optimal care of the HSCT recipient requires close monitoring paying particular attention to the emergence of infectious complications, to allow timely therapeutic interventions to be initiated to increase the chances of success in terms of quick recovery and survival.\textsuperscript{3} To achieve this, haematologists have to coordinate information from different sources besides their own e.g. the patient and his or her visitors as well as the professional carers including nurses, microbiologists, radiologists and other specialists. This can contribute to the timely discovery of a developing or progressive infection. However, in most hospitals, few of these people are present during the weekends and those that are, are often less experienced.\textsuperscript{4} This leads to breaches in the flow of information, and even to a lack of trust in the information given.\textsuperscript{5} As was shown in a survey on the treatment of neutropenic sepsis, this results in a delay in antibiotic administration, and hence, suboptimal care.\textsuperscript{6} Moreover, although unintended, patients may be placed at risk since diagnostic tests may be ordered out of anxiety rather than need and decisions may be based more on “gut feelings” than on objective criteria. This is potentially harmful to the patient as well as to the hospital ecology. Keuhlein et al. found fever during neutropenia will lead to a higher prescribing rate of antibiotics since the uncertainty was experienced as loss of control.\textsuperscript{7} De Pauw et al. showed that outside hospital hours, 3 out of every 4 decisions to modify antibiotic therapy during the first few days of fever in neutropenic patients were made without a reasonable explanation while 93% of similar decisions to change therapy during the normal working hours were made for objective reasons. Not only were these out-of-hours changes unnecessary, but they increased the risk of drug-related adverse events and led to unwarranted use of antibiotics.\textsuperscript{8} The so-called “weekend effect” poses an important problem when timing is crucial such as dealing with an acute pulmonary embolism or acute myocardial infarction\textsuperscript{9-11} since patients who are admitted during weekends have a significantly higher mortality in the short-term than do those admitted on weekdays.\textsuperscript{10,11} Since a HSCT can be planned and fever and infections can be predicted to a large extent as they tend to occur during the period of severe intestinal MBI that accompanies neutropenia following myeloablative conditioning it might be possible to avoid their occurrence altogether during weekends by clever planning.\textsuperscript{12,13} To explore this we undertook a retrospective analysis of patients with multiple myeloma (MM) who had been given high dose melphalan (HDM) in our department during 2008 to prepare
for an autologous HSCT as there were various supportive care trials running at the time which led to different days for starting HDM.

PATIENTS AND METHODS

Between January and December 2008 24 patients had been treated for MM with HDM. All had given their informed consent to the collection of data and plasma samples by participating in studies that had been approved by the local ethics committee (CMO Regio-Arnhem-Nijmegen). Each patient was managed with a 3 or 4 lumen central venous catheter (CVC) and received melphalan at a dose of 100 mg/m²/day on days -3 and -2 before transplant. Antimicrobial prophylaxis consisted of ciprofloxacin and valaciclovir. Each patient received a stem cell graft containing at least 2.0 x 10⁶ CD 34+ cells per kg on the day planned for transplantation. Patients received parental nutrition from the day of transplant onwards, but were not given any growth factors.

At the onset of fever, blood cultures were obtained, patients were examined for any sign of local infection, and empirical therapy with ceftazidime was started. Infections were defined according to the Consensus definitions of Immunocompromised Host Society. Data on neutropenia, intestinal MBI, inflammation and clinical and microbiological infections had been prospectively gathered from the day of starting chemotherapy until discharge.

NEUTROPHIL COUNT

The absolute neutrophil count (ANC) was determined daily and neutropenia was defined as an ANC ≤ 0.5 x 10⁹ /L.

INTESTINAL MUCOSAL DAMAGE

Plasma samples had been collected from 21 of the 24 patients to determine citrulline, an amino acid which blood concentrations directly reflect the functioning intestinal cell mass. This plasma was prepared and stored at −80°C for later analysis. Citrulline concentrations were measured by a standard procedure for determining amino acids using high-performance liquid chromatography (Shumadzu© Kyoto, Japan) and levels below 10 μmol/L were designated hypocitrullinaemia and considered to represent severe intestinal MBI.

C-REACTIVE PROTEIN (CRP)

CRP levels were determined every day until discharge using turbidimetric immunoassay (Aeroset©, Abbott). The day of maximum CRP and the highest level of CRP (CRP max) were recorded.
TEMPERATURE
The axillary temperature was measured 4-5 times a day and fever was defined by a single temperature of ≥ 38.5 °C. The maximum daily temperature was used for analysis.

BACTERAEMIA
Blood (10 ml) was drawn for aerobic culture each Monday and Thursday from each lumen of the CVC for surveillance. At the onset of fever, 40 ml of peripheral blood was obtained for culture together with 10 ml from each lumen of the CVC. Bacteraemia was defined by a single blood culture yielding any bacterial species except for coagulase-negative staphylococci which required the same isolate to be recovered from two separate blood cultures.¹⁴

DATA-ANALYSIS
We used descriptive statistics for each time point from the day of starting conditioning regime. Continuous variables are expressed as mean or median values together with a 95% confidence interval (CI). The categorical variables are displayed as absolute number and percentage. SAS version 9.2 software (SAS, Inc. Cary, NC, USA) was used for statistical analysis.

RESULTS
All 24 patients survived the first 6 weeks after transplant and none developed an invasive fungal disease. All patients had received melphalan at a dose of 200 mg/m² except for one individual who had received only 100 mg/m² because of kidney failure (this patient started with melphalan on a Tuesday and there were no samples collected for citrulline). Neutropenia started an average of 9 days (95% CI 8-10) after starting HDM and its mean duration was 9.5 (95% CI 7-10) days. All patients had elevated CRP levels exceeding 50 mg/L and the average peak value was 115 (95% CI 94-186) mg/L seen on average on day 15 (95% CI: 14-16). Eighteen (75%) of the 24 patients developed fever with a median day of onset of day 13 (95% CI 12-19). The mean duration of admission was 23.3 days (95% CI 21.7-24.8).
5 patients started with HDM on Tuesday, 8 started on Friday, 9 on Saturday and 2 on Sunday. All patients that started with HDM on a Tuesday developed fever at a weekend whereas none of those that started on Friday, Saturday or Sunday did so (Fisher’s exact test, P<0.001) (table 1).
12 (50%) patients developed bacteraemia, 5 due to oral viridans streptococci (OVS), 6 due to coagulase-negative staphylococci (CoNS) and 1 due to both bacteria. Of the patients with known citrulline values, those who developed bacteraemia also had hypocitrullinaemia at that time. 3 (60%) of the 5 patients who started HDM on Tuesday developed bacteraemia at the weekend (Table 1). The length of stay was the slightly longer for these patients, although the difference was not statistically significant (Table 2).
DISCUSSION

This study shows that the day of starting conditioning has implications for the occurrence of infections and inflammatory complications during the weekend as patients who started HDM on a Tuesday developed fever and bacteraemia more often during the weekend than did those starting on a Friday, Saturday or Sunday.

The 24 patients studied showed a very similar pattern regarding the occurrence of neutropenia, inflammation (course of CRP and occurrence of fever) and bacteraemia which is comparable with other reports. Since these studies investigated different patient cohorts who had received an autologous HSCT after conditioning with HDM in different hospitals, the only plausible explanation for the consistent pattern is that it is determined by the regimen given. Indeed, on average, these studies reported that fever occurred 12-13 days after starting HDM. Therefore it is not surprising that starting with HDM on a Tuesday is inviting trouble, since fever and infection will both occur during the weekend.

Fever occurred in 75% of our patients which is also in accordance with other studies and was accompanied by bacteraemia in about half of cases during hypocitrullinaemia as has been reported before in two other cohorts. The only bacteria recovered from blood cultures were CoNS and OVS which are residents of the alimentary tract especially the oral cavity and gut. This supports the contention that the GI MBI induced by the conditioning regimen provides a portal of entry to the systemic circulation by commensal flora. The use of ciprofloxacin as prophylaxis most likely gives a selective advantage to these Gram-positive cocci which are much less susceptible to the quinolone than are the enteric gram-negative bacilli associated with bacteraemia.

The period of increased risk of inflammatory and infectious problems can be predicted since every conditioning regimen appears to create its own pattern of occurrence of neutropenia and mucositis (Table 3). This suggests that similar predictions might be made for other conditioning regimens to select the best day for starting chemotherapy so as to have the best chance of avoiding infectious complications during the weekend.

If this is so, haematologists will have to change their current practice. At present, the day for starting treatment is dictated by many things including the wishes of the patients, nursing personnel, other specialists, or for logistic reasons. This study shows that since the haematologist can anticipate the occurrence of infectious complications they should decide the best day to start conditioning therapy for a HSCT. By reducing the risk for inflammatory and infectious problems during the weekend, HSCT recipients will be less exposed to the vagaries care during the weekends, which may, in turn, translate into better outcomes if, for example, antimicrobial therapy is started on time. In many cases unnecessary ordering of diagnostic tests may be reduced as can the unwarranted antibiotic use that exposes patients to side-effects and increases the risk of microbial resistance development. Furthermore, knowing infectious problems are likely to occur during the week makes managing HSCT recipients outside the hospital feasible and safe if accompanied by regular outpatient visits.
Table 3. Occurrence of fever (with or without bacteraemia) for different conditioning regimens

<table>
<thead>
<tr>
<th>Conditioning</th>
<th>Total pts</th>
<th>Type of cond.</th>
<th>Type of HSCT</th>
<th>Pts with fever</th>
<th>Fever, day</th>
<th>Bacteraemia on day fever</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N (%)</td>
<td>Mean (95% CI)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDM</td>
<td>56</td>
<td>MA Autologous</td>
<td>49 (88 %)</td>
<td>11.8 (11.4-12.2)</td>
<td>29 (52 %)</td>
<td></td>
</tr>
<tr>
<td>BEAM</td>
<td>21</td>
<td>MA Autologous</td>
<td>19 (90 %)</td>
<td>13.0 (12.2-13.9)</td>
<td>10 (48 %)</td>
<td></td>
</tr>
<tr>
<td>Ida-Cyclo-TBI</td>
<td>28</td>
<td>MA Matched sibling allogeneic</td>
<td>28 (100 %)</td>
<td>12.9 (12.2-13.6)</td>
<td>18 (64 %)</td>
<td></td>
</tr>
<tr>
<td>Cyclo-ATG-TBI</td>
<td>34</td>
<td>MA Matched sibling allogeneic</td>
<td>Early: 25 (73.5 %)</td>
<td>4.1 (3.7-4.5)</td>
<td>29 (85 %)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Late: 34 (100 %)</td>
<td>14.1 (13.0-15.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclo-TBI</td>
<td>10</td>
<td>MA Matched sibling allogeneic</td>
<td>10 (100 %)</td>
<td>13.4 (12.0-14.8)</td>
<td>6 (60 %)</td>
<td></td>
</tr>
<tr>
<td>Cyclo-Flu</td>
<td>14</td>
<td>NMA Matched sibling allogeneic</td>
<td>Early: 4 (28.6 %)</td>
<td>3.0 (1.7-4.3)</td>
<td>0 (0 %)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Late: 11 (78.6 %)</td>
<td>16.0 (14.4-17.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adapted, with permission, from Van der Velden et al.\textsuperscript{19}

Every conditioning regimen has its own pattern of occurrence of fever. In some of the patients treated with Cyclo-ATG-TBI and Cyclo-Flu there were two episodes of fever (early and late episode). Not all patients that develop fever have also bacteraemia.

**CONCLUSION**

Better planning of the HSCT will improve the chances of avoiding fever and bacteraemia during the weekend. This would help limit unwarranted use of antibiotics and the unnecessary ordering of diagnostic tests thereby lowering the risk of adverse events related to drugs or unnecessary diagnostic tests, the selective pressure for antibiotic resistance, as well as reducing health care-related costs.

**REFERENCES**


CHAPTER 11

MBI, FEVER AND INFECTION IN NEUTROPENIC PATIENTS WITH CANCER, INTRODUCING THE PARADIGM FEBRILE MUCOSITIS

ABSTRACT

Infection remains one of the most prominent complications after cytotoxic treatment for cancer. The connection between neutropenia and both infections and fever has been designated “febrile neutropenia” decades ago. However, treatment with antimicrobial agents and hematopoietic growth factors has failed to significantly reduce the incidence of febrile neutropenia. Moreover, emerging antimicrobial resistance is becoming a concern which necessitates the judicious use of available antimicrobial agents. Besides neutropenia, patients who receive cytotoxic therapy experience mucosal barrier injury (MBI) or “mucositis”. MBI creates a port-de-entrée for resident micro-organisms to cause blood stream infections, and contributes directly to the occurrence of fever by disrupting the highly regulated host-microbe interactions, which, even in the absence of an infection, can result in strong inflammatory reactions. Indeed, MBI has been shown to be a pivotal factor in the occurrence of inflammatory complications after cytotoxic therapy. Hence, the paradigm “febrile neutropenia” alone may no longer suffice and the new paradigm “febrile mucositis” is more appreciated as the two paradigms are complementary. In this review we summarise the existing evidence for both paradigms and propose new therapeutic approaches to tackle the perturbed host-microbe interactions arising from cytotoxic therapy-induced tissue damage in order to reduce fever in neutropenic patients with cancer.
INTRODUCTION

Infection is a common complication during the neutropenia that accompanies cytotoxic therapy used to treat patients with solid and hematological malignancies.\(^1\) Fever is often the first and only sign of infection and represents a signal to start empirical antibacterial therapy promptly, so as to reduce the chance of fulminant sepsis due to gram-negative bacillary infection and death.\(^2\)\(^-\)\(^5\) Additional empirical antifungal therapy is often initiated in case of persistent fever, but invasive fungal infection comprise at most 5-10% of febrile episodes.\(^6\)-\(^8\) However, fever remains unexplained in 30-50% of neutropenic patients, as there is no evidence found of a clinically or microbiologically defined infection.\(^9\) Furthermore, irrespective of the aetiology, fever persists for 4-5 days or even longer in a substantial number of cases (approximately 30%) despite adequate antimicrobial therapy directed at bacteria and fungi.\(^10\)\(^-\)\(^12\) This suggests that fever is not necessarily related to infection. Indeed, it is often not appreciated that fever is a nonspecific response to a variety of insults of which infection is only one. Living microorganisms and the microbial wall motifs (pathogen-associated molecular patterns (PAMPs)) are powerful inducers of cytokine release.\(^13\),\(^14\) A similar role has been proposed for the danger-associated molecular patterns (DAMPs) that are released from damaged tissues.\(^15\) Indeed there are many inflammatory diseases that are, in essence, a manifestation of a perturbed host-response in the face of “damage”.\(^16\) Most of these diseases occur at the integument where epithelial and mucosal barriers are inhabited by micro-organisms. At the mucosal level, there is strict control and regulation of host responses especially when confronted with tissue damage, in order to prevent infection on the one hand, and overwhelming inflammation on the other hand.\(^17\),\(^18\)

Patients who become neutropenic after cytotoxic therapy also suffer simultaneous disruption of the integument along the entire alimentary tract including the oral cavity.\(^19\) The “mucosal barrier injury (MBI)” itself plays a major role in the occurrence of infection and fever. Perturbation of the mucosal barrier results in a port-de-entrée for certain micro-organisms to invade the body and cause local infection and bacteraemia. Moreover, various DAMPs and PAMPs are released, all of which are able to elicit a powerful inflammatory response that results in fever. Indeed several studies have suggested MBI to be more important than neutropenia in causing infectious complications after chemotherapy and haematopoietic stem cell transplantation (HSCT).\(^20\),\(^21\) Therefore, the paradigm “febrile neutropenia” alone no longer suffices as fever may well be a response to MBI whether or not infection is involved.

This led us to critically appraise the concept of “febrile neutropenia” and review the evidence that MBI plays a more important role than does neutropenia per se in the aetiology of fever and infection. Important consequences may arise from this paradigm shift because therapeutic approaches other than the use of antimicrobial agents may prove efficacious and enable restrictive use of antimicrobial agents in a time of emerging antimicrobial resistance.
Chapter 11

FEBRILE NEUTROPENIA

1. THE CONCEPT OF FEBRILE NEUTROPENIA

In 1966, Bodey et al. published a landmark article on the quantitative relationship between neutrophils and infection in patients given chemotherapy to treat acute leukaemia.1 This relationship held for a variety of proven infections ranging from local infection, e.g. pneumonia, to disseminated infection, e.g. bacteraemia, hence introduction of the paradigm “febrile neutropenia”. However, the occurrence of fever of unknown origin (FUO) appeared not to be closely related to neutropenia suggesting that the lack of neutrophils was not the sole determinant of fever.

Fever that occurs during neutropenia probably results from the release of cytokines by residual immune cells and stromal cells, e.g. epithelial cells, that sense micro-organisms and molecular motifs through their pattern-recognition receptors (PRRs). Also other pathophysiological mechanisms have been proposed to mediate the occurrence of “febrile neutropenia”. One of these hypotheses suggests that a subset of neutrophils are anti-inflammatory as they elaborate IL-10,22 whereas other neutrophils normally produce powerful anti-inflammatory molecules like antimicrobial peptides and cytokine antagonists, including IL-1 receptor antagonist (IL-1Ra), that can attenuate inflammation.23 Lower levels of these molecules will prevent negative-feedback to inflammation which is a natural protective mechanism from damage.24 Another possible explanation might be the increased levels of uric acid during neutropenia, which leads to increased responsiveness of innate immune cells to microbial stimulation.25

2. ORIGIN OF INFECTIONS DURING NEUTROPENIA

The host defences are profoundly affected and mucosal barriers are damaged as a result of the treatment of cancer using chemotherapy and radiotherapy. Consequently, infections are typically due to those opportunistic pathogens that inhabit the skin and oral and gastrointestinal tract rather than professional pathogens such as Streptococcus pneumoniae.26-28 By contrast, the incidence of infection is much lower in chronic idiopathic neutropenia and untreated hematological malignancies and infections are predominantly due to the professional pathogens that affect the respiratory tract and skin.29,30 The incidence of FUO and bacteraemia is low which can partly be explained by the absence of chemotherapy-induced MBI.29

2.1 Prophylactic antimicrobial therapy for afebrile neutropenic patients

Antimicrobial chemoprophylaxis is commonly given to patients receiving intensive cytotoxic therapy and recipients of a HSCT who are expected to suffer from profound neutropenia.31 Indeed, the use of certain fluoroquinolones for this purpose improves overall survival.32-34 The evolution of antimicrobial prophylaxis began with the simple aim of decontaminating the gut to prevent the translocation of gram-negative bacilli that resided in the intestinal tract such as Escherichia coli that most often caused infection.11 Fluoroquinolones, particularly
ciprofloxacin and levofloxacin, are ideally suited to this purpose as they prevent the occurrence of gram-negative bacilliary bacteraemia, although their clinical use has been increasingly questioned because of the emergence of resistance and concerns regarding increased risk for infections with *Clostridium difficile*. Moreover, most patients on prophylaxis still become febrile with only about 1 in 3 being explained by documented infections.

2.2 *Empirical antimicrobial therapy for febrile neutropenia*

Empirical use of broad-spectrum antimicrobial agents to manage febrile neutropenia has become the standard of care and has resulted in improved survival in cancer patients. However, the rate of FUO has not been reduced substantially and a large minority of patients remain febrile despite broad-spectrum antimicrobial therapy. Almost 2 in 3 patients and 1 in 3 patients are still febrile respectively 4 days after the onset of fever. Many clinicians assume the presence of cryptic infection and change the antimicrobial regimen empirically, but to little avail as more than half of patients who respond to antibiotics are still febrile after 3 days.

3. **COLONY-STIMULATING FACTORS AND GRANULOCYTE TRANSFUSION**

The relationship between neutropenia and infections drove the development of hematopoietic growth-factors such as granulocyte (monocyte) colony-stimulating factor (GM-CSF), and granulocyte transfusions, with the expectation of lowering the incidence of infections by reducing the duration and depth of neutropenia. Indeed CSFs were shown to marginally reduce infection-related mortality, as well as shorten the duration of neutropenia. However, their use is not favoured for patients with protracted neutropenia following intensive chemotherapy as the duration of neutropenia is only abridged by a few days, and the modest clinical benefit in reducing infections and the marginal reduction in mortality does not outweigh the costs and adverse effects. In addition, there is little if any clinical benefit for granulocyte transfusions and there are even reports suggesting a detrimental impact on the outcome of infection.

Therefore, it would seem reasonable to seek an alternative explanation for fever as it occurs despite effective suppression of bacterial infections using prophylaxis, it persists despite broad-spectrum antimicrobial therapy, an infectious aetiology is seldom identified and the use of growth factors fails to reduce fever and infections significantly.

**FEVER RELATED TO MUCOSAL BARRIER INJURY**

Several diseases besides infections are accompanied by fever, including the cancer itself and drug reactions. In 1984 it was already suggested that intestinal damage caused by chemotherapy might result in endotoxinaemia leading to fever. This hypothesis is in line with the fact that most bacteria found in blood cultures originated from the gut flora that translocated through
ulcerated barriers. Moreover it was generally believed that oral mucositis coincided with neutropenia and fever but had no causal relationship. This belief changed as what became known as MBI began better understood.

1. PATHOGENESIS OF MBI AND RELATED FEVER
The pathogenesis of MBI is thought to consist of five phases:\n\[19\]: 1) the activation of nuclear factor-kappa B directly by chemotherapy and radiotherapy and indirectly from formation of reactive oxygen species, DNA, and non-DNA damage\[46\], 2) production and release of cytokines and chemokines (IL-1β, IL-6, IL-8, TNFα, IL-23, interferon-gamma (IFNγ) by tissue macrophages, dendritic cells, and epithelial cells\[^{47,48}\] , 3) positive feedback loop of TNFα, epithelial cell apoptosis and increased mucosal permeability\[^{49}\] , 4) translocation of microbes and PAMPs aggravating inflammation\[^{50-52}\] , and 5) repair and healing. Most data on the pathogenesis of MBI come from animal studies, but it is likely that the model also explains human complications. Although developed to explain oral mucositis, the model can probably be extended to gastrointestinal mucositis.\[^{53}\]

It is thought that MBI is essentially an inflammatory disorder that results from perturbation of the normal innate immune system at the mucosal barriers, leading to distorted host-microbe interactions (Figure 1).\[^{16}\] The sequence of events in the gut of mice show that chemotherapy induces an initially sterile inflammatory response at the mucosal level of the gut, which is then followed by changes in the epithelium, apoptosis resulting in crypt hypoplasia, and villous blunting which, in turn, leads to a breach in the barrier.\[^{48,52,53}\] MBI is established before translocation of bacteria and PAMPs occurs, which, in turn, enhance the response of the already primed innate immune cells that reside in the tissue. This culminates in a systemic inflammatory response syndrome that manifests itself primarily as fever and is associated with other complications such as the non-cardiogenic pulmonary edema, idiopathic pneumonia syndrome, engraftment syndrome, and graft-versus-host disease (GvHD).\[^{54}\] Therefore, what is known as “febrile neutropenia” can better be described as “MBI-related fever” as it summarizes better the pathological connection between MBI and fever, whether or not infection occurs.
Introducing the paradigm febrile mucositis

In health there is a tightly controlled immune balance at the mucosal barriers with tolerance to commensal flora, and absence of tissue damage. PRRs sensing PAMPs play an import role in maintaining this homeostasis. The microbial flora is kept in check, for instance through the release of antimicrobial peptides (AMPs), and immune responses are dampened by preservation of the physical barrier, selective expression of PRRs, and beneficial properties of commensal microorganisms. There is a low state of immune activation and inflammation (‘physiological inflammation’) that contributes to the development of a healthy local and systemic immune system and keeps the immune system on stand-by (left panel). During health, innate immune responses modulate the acquired immune responses towards a tolerogenic profile contributing to the homeostasis.

Due to MBI resulting from cytotoxic therapy and mucosal damage resulting from GvHD the mucosal barriers and immune homeostasis are perturbed. Microorganisms and PAMPs are able to translocate and induce inflammation by activating PRRs. This is aggravated by the release of DAMPs released on the occurrence of tissue damage. The microbial flora is not kept in check with an increase in pathogenic microorganisms (dysbiosis) (right panel). Deregulated innate immune responses contribute to the development of inflammatory complications by eliciting pro-inflammatory acquired immune responses which can further damage the mucosal barriers. Tine Thörig scientific illustrations.

TSLP = thymic stromal lymphopoietin, TGFβ = transforming growth factor beta, HMGB-1 = high mobility group box 1, HSP = heat shock protein, LPS = lipopolysaccharide, LTA = lipoteichoic acid, MDP = muramyl dipeptide and AMP = antimicrobial peptide.
1.1 Innate immunity and MBI
During MBI, the stimulation of PRRs by invading micro-organisms and PAMPs that have translocated through the disrupted mucosal barrier appear to aggravate inflammation and fever. However, data on the role of perturbed innate host-microbe interactions during MBI come mainly from GvHD studies. The tissue damage following conditioning therapy for allogeneic HSCT that constitutes the initiation phase of acute GvHD mostly involves gastro-intestinal MBI. The inflammation that ensues causes activation of host antigen-presenting cells that enables them to initiate and perpetuate the allo-immune responses that account for acute GvHD. The role of endotoxins translocating from the gut in activating the immune system through Toll-like receptor (TLR)-4 in GvHD was postulated 20 years ago. TLR9 activation may also play a pivotal role in GvHD, mainly through intestinal epithelial cells being activated by microbial DNA.

In addition, during MBI the innate immune system is activated by DAMPs in a similar fashion as PAMPs. DAMPs are released by cells that are killed by chemotherapy and radiotherapy and include ATP, high-mobility box group-1 (HMGB-1) and heat-shock proteins. The process is designated ‘immunogenic cell death’ because these molecules mediate immune responses. Among cytotoxic therapies, anthracyclines, cyclophosphamide and radiotherapy are strong inducers of immunogenic cell death by inducing apoptosis and necrosis of epithelial, endothelial and other cells (Figure 1). Subsequently, they activate tissue macrophages, and stromal cells, through the activation of PRRs, including TLR4 and the P2X7 receptor. DAMPs have also been suggested to be involved in mucosal damage-related inflammation in HSCT.

Single nucleotide polymorphisms (SNPs) in innate immune genes in the setting of HSCT also suggest a role for innate immunity in MBI. The best-known examples are the SNPs of the nucleotide-oligomerization domain peptide (NOD)-2, a PRR that plays a pivotal role in mucosal barrier defences. NOD2 SNPs result in reduced microbial clearance, increased gut permeability, and uncontrolled inflammation. NOD2 SNPs have been associated with the occurrence of Crohn’s disease as well as severe acute GvHD. This effect is related to the severity of intestinal MBI and the use of T cell-depletion.

Innate immune signalling induced by changes in the gut microbial flora (microbiota) during conditioning-induced MBI influences GvHD. Modification of the gut microbiota also alters the course of acute GvHD in mice indicating a clear role for bacterial communities in GvHD. Clinical studies of HSCT recipients have also shown that the use of ciprofloxacin and metronidazole markedly reduced the incidence and severity of GvHD. More recently, the role of the microbiota has also been shown of importance in MBI outside the setting of allogeneic HSCT.

1.2 Anti-inflammatory properties of epithelial cells
Besides preventing the invasion of micro-organisms by forming a physical barrier, epithelial cells have other ways of preventing inflammation in the presence of overwhelming numbers of microbial residents. These comprise three major groups of molecules; 1) antimicrobial
Introducing the paradigm febrile mucositis

peptides, 2) anti-inflammatory cytokines including IL-10, and transforming growth factor β (TGFβ), and 3) cytokine receptor antagonists, including IL-1Ra. Damage inflicted on epithelial cells can therefore disrupt tightly regulated anti-inflammatory mechanisms in addition to the loss of the barrier function. Losing the ability to resolve an inflammatory response by deficiencies in these molecules will eventually contribute to aggravated tissue damage, inflammation and fever.

2. CLINICAL CONSEQUENCES OF MBI

2.1 MBI, infection, inflammation and fever
Several studies have shown the occurrence of MBI to have a major impact on complications of cancer therapy and, albeit indirectly, illustrated the influence of MBI on fever. The report of Sonis et al. in 2001 showed that the number of days with fever and early infections in HSCT recipients increased proportionally with the highest mucositis score. Similar results were found among patients with solid tumours and lymphoma with MBI being independently associated with FUO and infection. MBI is also associated significantly with greater use of total parental nutrition and opioid analgesics, as well as increased costs, length of hospital stay, and mortality. The more recent Prospective Oral Mucositis Audit found that a high incidence of oral MBI corresponded to different clinical and economic outcomes in autologous HSCT recipients. Chemotherapy-induced MBI is associated with early onset bacteraemia. Before the introduction of antimicrobial prophylaxis, gram-negative enteric bacilli predominated as causes of infection. Later on, the gram-positive cocci particularly the oral viridans streptococci (OVS), and coagulase-negative staphylococci (CoNS) prevailed. The streptococci reside on the mucosal barriers and their numbers increase as a result of antimicrobial prophylaxis. It is widely assumed that the CoNS bacteraemia results from this skin commensals invading the bloodstream via central venous catheters, but this assumption has been challenged as CoNS also reside on mucosal barriers. Indeed Costa et al. typed CoNS isolates retrieved from skin, gut and the bloodstream and found that the gut mucosa was the most likely source of these bacteria. Gut microbes reach the bloodstream by traversing the mucosa of the gut which shows signs of hyperpermeability among these patients. Other factors, including the composition of the microbial flora, bacterial overgrowth, and concomitant immune dysfunction, seem also to be required. However, following cytotoxic therapy the relationship between gut permeability and bacterial translocation is fairly consistent, probably because most patients are neutropenic the moment MBI occurs, and therefore the second line of defence consisting of phagocytic cells is no longer able to prevent translocation. The peak of permeability of the gut has been shown to occur 10-14 days after starting the intensive chemotherapy. Bow et al. found a direct relationship between D-xylose malabsorption and invasive bacterial and fungal infections of patients with acute myeloid leukaemia with the highest incidence occurring in the second week after starting chemotherapy when there was marked malabsorption. Similarly,
several studies have shown that bacteraemia occurs on average 12-14 days following high-dose chemotherapy (Figure 2). The timing also correlates with the development of severe intestinal MBI as determined by sugar malabsorption and low citrulline levels.

The relationship between MBI and the inflammatory response and fever among patients receiving an autologous HSCT showed inflammation clearly coincided with the occurrence and progression of oral and intestinal MBI (Figure 2). Fever occurred 12 days after the start of conditioning treatment and coincided with the presence of severe intestinal damage regardless the presence or absence of bacteraemia. This pattern was similar to that seen among patients receiving chemotherapy for acute myeloid leukaemia. These observations were confirmed in a subsequent study of 6 different conditioning regimens. Once again a clear pattern of inflammation and fever was observed that coincided with the progression of MBI. There was a strong correlation between the extent of intestinal MBI and the evolution of C-reactive protein; a biomarker of inflammation that correlates with pro-inflammatory cytokines.
Introducing the paradigm febrile mucositis

such as IL-8. Comparing patients who had received a non-myeloablative conditioning versus patients treated with a myeloablative regimen showed that in the non-myeloablative conditioning very little intestinal mucositis was accompanied with limited inflammation and a lack of bacteraemia, despite profound neutropenia. This provides evidence that the presence or absence of MBI determines to a large extent the early complications seen after HSCT. Moreover the occurrence of the so-called “alpha-streptococci syndrome” which is characterised by bacteraemia due to OVS, and septic shock or non-cardiogenic pulmonary oedema, was related to the degree of underlying MBI. Interestingly, it was already noted decades ago that severe oral mucositis rather than infection appeared to induce an acute-phase response with fever suggesting bacteraemia due to these OVS to have only been a consequence of mucosal damage and not the primary initiator of the inflammatory response. This is consistent with the observation that the occurrence of pulmonary oedema correlated strongly with cytarabine-induced intestinal lesions. Hence it would appear that the host-microbe interaction defines the outcome of OVS bacteraemia rather than the opportunistic pathogen alone, as it exhibits low virulence. Therefore, restoring the balance by ameliorating the inappropriate host response might prove beneficial and improve the outcome of these patients.

2.2 Mucosal barrier preservation ameliorates complications

The concept of “MBI-related fever” is supported by the consistent observation that reducing mucosal damage attenuates fever and infection. The pioneering study of Spielberger et al. showed that recombinant human keratinocyte growth factor (rh-KGF, Palifermin®) significantly reduced the incidence of oral mucositis in patients receiving a HSCT after conditioning containing total body irradiation. The incidence of febrile neutropenia was also significantly decreased, by almost 20%, although the drug had no effect on the duration of neutropenia. Exploratory analysis also showed a trend towards a lower incidence of bacteraemia in the KGF group than in the placebo group (15 versus 25 percent). Similarly, the use of recombinant human IL-11 preserved the gut barrier after cytotoxic therapy and resulted in a decrease in bacteraemia. Comparable results have been shown with the use of less mucotoxic therapies such as the so-called reduced-intensity conditioning regimens, which result in fewer days of fever and less gram-positive coccal bacteraemia.

The association between chemotherapy-induced MBI of the gut and the occurrence of acute GvHD, which frequently involves the gut is compelling. Not surprisingly, by preserving the mucosal barrier using less mucotoxic non-myeloablative conditioning regimens and drugs including rh-KGF and Wnt signalling agonist R-spondin-1, has been shown to ameliorate or delay acute GvHD, although thus far only in animal studies. In humans rh-KGF seemed not protective for GvHD, despite the reduced incidence of oral mucositis, but this is probably explained by a virtual lack of effect of the growth factor on intestinal mucositis.
3. CONTEXT DEPENDENT ROLE FOR MBI?

It is important to realise that the extent of MBI differs strongly among patient categories, cancer subtypes and mostly the cytotoxic therapies that are employed. Intensive cytotoxic therapies that are used for hematological malignancies bear the highest risk for MBI and related inflammatory complications. The exact role for MBI in the treatment of solid tumours is less clear and warrant further investigation. Nevertheless, most chemotherapy schemes accompanied by a 20% or higher incidence of febrile neutropenia are associated with the occurrence of mucositis and also in these setting MBI may contribute to the occurrence of fever.96,97

RESEARCH QUESTIONS

1. NEW WAYS OF TREATING FEVER IN CANCER PATIENTS?

The concepts of febrile neutropenia and MBI-related fever are at least complementary and have implications for the care of neutropenic patients at risk for developing fever and infection, not least in reassessing the role of antimicrobial strategies (Figure 3). Antibacterial prophylaxis may be most beneficial to patients who develop MBI. When fever develops, switching to parenteral antibiotics empirically is common practice although this may not always be effective or even necessary in all patients. However, it cannot be predicted who is to benefit and who is not and therefore empirical antibiotics cannot be discarded. Nevertheless, simply switching or adding antimicrobial agents for persisting FUO should be discouraged, although in the absence of diagnostic tools or a high background incidence of fungal infections empirical antifungal therapy might be considered. Instead, especially because of the growing armamentarium of diagnostic tools, an attempt should be made to diagnose microbiologically and clinically defined infections. Further therapy could then be directed by the findings. This would limit antimicrobial therapy to those most at risk (Figure 3) and would help limit the emergence of antimicrobial resistance, reduce the risk of inducing dysbiosis and help contain health costs. The modest effects of hematopoietic growth factors should also be carefully balanced against the potential side effects and high costs.
Introducing the paradigm febrile mucositis

Patients treated with chemotherapy for cancer can mount a sustained inflammatory response with fever irrespective of whether an infection is involved. This is partly the result of MBI of the oral cavity and gut. The innate immune responses during this process are not necessarily directed at any particular microbial pathogen, but can be initiated by microbial motifs (PAMPs) and tissue damage (DAMPs) alone. The resulting fever leads to therapy with antimicrobial agents, which in a substantial proportion of patients will have no effect on the persisting fever and inflammation. Therefore other approaches should be explored, including the use of mucosal growth factors, PRR agonists and antagonists, and pre- and probiotics, in order to restore the perturbed host microbe responses at the integument so the outcome of cancer patients can be improved.

Other approaches might be more appropriate for dealing with protracted inflammation and fever given the role of MBI and innate immune perturbations. For instance, restoring mucosal health or at least preventing mucosal damage of the oral cavity and gut can avert microbial translocation, bacteraemia, and innate immune activation by PAMPs. However, there are few remedies available to ameliorate MBI.98 Borrowing ideas from other areas with knowledge of MBI and deregulated immunity, for instance gastroenterology (inflammatory bowel diseases) and hematology (GvHD), may help. Promising approaches include the use of growth factors to stimulate intestinal stem cells, e.g. R-spondin-1, IL-22 and glucagon-like peptide,99 modulation of PRR activation by agonist and antagonists,100 and modulation of the gut microbiota.101 The microbiota can also be modulated by employing pre- and probiotics and has been shown effective in treating antibiotic-associated diarrhoea caused by Clostridium difficile and gut inflammation.102 Guidelines for managing intestinal mucositis already suggest that probiotic treatment containing Lactobacillus species may prevent chemotherapy and radiotherapy-induced diarrhoea in patients with pelvic malignancies.98

Exploiting the existing negative feedback and anti-inflammatory pathways that facilitate tissue
repair and resolution of inflammation might offer another approach.\textsuperscript{103} This can be achieved by correcting deficiencies in antimicrobial peptides, e.g. lactoferrin and defensins,\textsuperscript{104} using anti-inflammatory cytokines e.g. IL-1\textsubscript{0}, and with the use of cytokine inhibitors, e.g. IL-1Ra.\textsuperscript{105} Similarly, scavengers of PAMPs and DAMPs may prove productive to study with candidate molecules consisting of antimicrobial peptides, innate defence regulators, and recombinant antibodies, for example anti-HMGB-1.\textsuperscript{106}

2. RISK PREDICTION AND INDIVIDUALIZED RISK?

It would be very helpful if the risk of complications could be estimated before starting treatment of malignancies. This would require the integration of patient characteristics (age, gender), treatment characteristics (type of chemotherapy, schedule, intensity), and genetic predisposition. Genes related to drug metabolism, organ function, host immunity, tissue repair and stem cell recovery are all likely to be involved in the development of neutropenia, MBI, and infection. For instance, relationships have been shown between TLR4 polymorphisms and neutropenia in children with acute lymphoblastic leukaemia,\textsuperscript{107} polymorphisms in mannose binding lectin, TLR4 and dectin-1 and an increased risk of bacterial and fungal infections\textsuperscript{108,109} and NOD2 polymorphisms have been associated with Crohn’s disease and GvHD.\textsuperscript{64} Citrulline has been shown to be a good marker of gut damage and permeability with low levels being associated with the occurrence of bacteraemia and inflammation.\textsuperscript{21,83} Other potential biomarkers include regenerating islet-derived 3-alfa, as it correlates well with the severity of intestinal GvHD,\textsuperscript{110} and intestinal fatty acid-binding protein and ileal bile acid-binding protein, both proteins being released by dying mature enterocytes.\textsuperscript{111}

The old idea of preserving the gut ecology has been given a new impulse with recent advances in molecular biology that allow the identification of the composition of the patients own gut microbiota. Hence we now know that there are three different enterotypes\textsuperscript{112} and that dysbiosis has important impact on auto-immunity, allergy and auto-inflammatory diseases. There are also strong indications that the microbiota varies amongst patients with cancer and has an impact on the occurrence of MBI and the related inflammatory response.\textsuperscript{66,69} Moreover, oral and colonization with \textit{Candida} species have been related to the occurrence of GvHD, especially of the gut.\textsuperscript{113} This knowledge might be used in the future to better predict which patients are at most risk for developing chemotherapy-induced MBI and related complications.

CONCLUSION

Patients treated with chemotherapy for cancer can mount a sustained inflammatory response with fever irrespective of whether an infection is involved. This is partly the result of MBI of the oral cavity and gut. The innate immune responses during this process are not directed at any particular microbial pathogen but are initiated by microbial motifs (PAMPs) and tissue
Introducing the paradigm febrile mucositis
damage (DAMPs) and are excessive, inappropriate and propagate tissue damage resulting in 
an increased burden of illness for cancer patients. The resulting fever is currently managed by 
employing antimicrobials for therapy, which in certain sub-categories of patients has little or no 
effect on persisting fever and inflammation. The time seems ripe for considering an alternative 
model that takes MBI and innate immunity into account and complement the paradigm of 
“febrile neutropenia” with that of “febrile mucositis”. This should provide a new impulse for 
developing drugs and other therapeutic approaches to tackle the principal problem and 
re-establish the balance between the host and the microbiota.

REFERENCES

1. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection 
3. Schimpff S, Satterlee W, Young VM, Serpick A. Empiric therapy with carbenicillin and gentamicin for febrile patients 
4. Sickles EA, Greene WH, Wiernik PH. Clinical presentation of infection in granulocytopenic patients. Arch Intern 
5. Wright JD, Neugut AI, Ananth CV et al. Deviations from guideline-based therapy for febrile neutropenia in cancer 
6. Cornely OA, Maertens J, Winston DJ et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients 
7. Klastersky J. The changing face of febrile neutropenia - from monotherapy to moulds to mucositis. Why empirical 
8. Kontoyiannis DP, Marr KA, Park BJ et al. Prospective surveillance for invasive fungal infections in hematopoietic 
stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network 
Chemother 1993; 31: 813-829.
13. Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal 


48. Logan RM, Stringer AM, Bowen JM, Gibson RJ, Sonis ST, Keefe DM. Is the pathobiology of chemotherapy-induced


Introducing the paradigm febrile mucositis


105. Carvalho FA, Aitken JD, Gewirtz AT, Vijay-Kumar M. TLR5 activation induces secretory interleukin-1 receptor antagonist (sIL-1Ra) and reduces inflammasome-associated tissue damage. Mucosal Immunol 2011; 4: 102-111.


Chapter 12

SUMMARY

Studying the role of citrulline in HSCT recipients is the focus of this thesis. Citrulline is a marker of cytotoxic therapy-induced intestinal mucosal barrier injury (MBI) allowing us to explore the role of intestinal MBI in the occurrence of fever and infections. The value that measuring citrulline could add to the current care of the HSCT recipient is discussed.

PART I: BACKGROUND OF MANAGING FEBRILE NEUTROPENIA IN HEMATOLOGY PATIENTS

In this first part it is described how historical perspectives still determine the approach of fever and infections in the current daily practice in patients with hematological malignancies. Until 1960’s, most patients with cancer could not be cured but this began to change after the early successes of cytotoxic chemotherapy. However it became rapidly clear that the destructive effect of chemotherapy was not limited to cancer cells. Haemorrhage and infection emerged as the most prominent complications of chemotherapy. Blood transfusion was an effective remedy for bleeding but infection proved more intractable as it was difficult to diagnose early enough to treat effectively, and such antibiotics as were available were limited in spectrum, had to be given in combination, and were only marginally effective. By 1966 a positive correlation was shown between the severity and duration of neutropenia (defined as an absolute neutrophil count of ≤ 0.5 x 10^9 granulocytes/L) and the risk of acquiring a life-threatening bacterial infection. Since fever in the immunocompromised patient was most often the first and only sign of infection, the syndrome became known as “febrile neutropenia” to indicate a life-threatening event that, if left untreated, could result in fulminant sepsis and death. A few years later it was demonstrated convincingly that lives were saved by administering promptly antimicrobial agents that covered the common pathogens. This approach became known as empirical therapy which was given at the onset of fever during neutropenia without waiting for the results of blood cultures. As a result, it was widely adopted and soon became the standard of care. In the years that followed, better antimicrobial agents allowed doctors to intensify their chemotherapy regimens. A spiral of subsequent rounds of improved antibiotic cover and further escalations of cytotoxic regimens ensued resulting in more remission of hematological malignancies and in helping stem cell transplantation to become successful enough to become an accepted treatment modality.

The approach to preventing and treating infectious complications remained essentially the same and emphasised neutropenia as the primary risk factor. Hence physicians and nurses aligned their monitoring and care to neutropenia to detect infections as early as possible so that empirical antibiotic treatment could be started as soon as fever developed. The practice of empirically adjusting initial empirical therapy also became widespread with a variety of algorithms being adopted for treating febrile neutropenic patients without an evident infectious focus. Typically these algorithms dictated that adjustments to the empirical antimicrobial
therapy should be made after a fixed duration of fever typically three days after onset, even if the patient was stable or recovering. Moreover, antimicrobials were to be given for at least 7 days or more or until neutropenia resolved.

This placed a great demand on the antimicrobials that were available leading to the overuse of these agents but was justified on the grounds that a broader antimicrobial coverage would benefit the patient. The downside was largely ignored but included induction of antimicrobial resistance, perturbation of the ecological microbial balance of the gut flora as well as drug-induced side effects that might include drug fever, rash or even organ toxicity.

In fact, antimicrobial resistance and the sharp decline in the development of new antimicrobial agents now pose a serious threat to caring for these patients in the future. This is reason enough to reconsider our current strategies and revisit the impact of the cytotoxic therapy-induced damage to other parts of the host defences (chapter 2).

PART II: INTRODUCTION OF CITRULLINE AS BIOMARKER FOR INTESTINAL MUCOSAL BARRIER INJURY

To investigate the role of intestinal MBI in the development of fever and infections, it is important to have a reliable biomarker. The amino acid citrulline is one of the new potential biomarkers of intestinal MBI and was studied in clinical studies.

Chapter 3 is a review of the amino acid citrulline and its potential role as a biomarker for MBI. Citrulline is not incorporated into proteins which means that circulating citrulline in the blood depends only on “de novo” synthesis and absorption of food. In the case of citrulline, it is not only not commonly present in food, but it is almost exclusively synthesized and released by the intestine (especially the small bowel) into the bloodstream. Hence, irrespective of the cause, damage to the intestine will result in lower levels of circulating citrulline. Citrulline is not influenced by inflammation, there is no appreciable uptake by the liver and the kidney is the major consumer of the amino acid. Accordingly, with normal renal function, the concentration of citrulline depends only on the production and release of citrulline by the intestine. Furthermore citrulline can be measured by a simple and relatively inexpensive blood test. The review shows that the use of citrulline enables standardized assessment of intestinal MBI which can improve the management of intestinal MBI- related complications.

In chapter 4 plasma citrulline was assessed as marker for intestinal MBI in a cohort of 94 recipients of a HSCT who had received different conditioning regimens. It was shown that each treatment regimen induced a unique and different course of MBI. Two citrulline-based assessment scores were developed; one based on the level of citrulline, using severity thresholds, and a second based on the area under the reciprocal (10/citrulline) curve. Both are able to delineate between damage induced by different conditioning regimens. However the score based on the area under the reciprocal curve of citrulline is probably only appropriate for research purposes, though only a few measurements are necessary to estimate this score. For
clinical purposes, a scoring system based on absolute citrulline concentrations seems more practical for determining intestinal MBI. This makes a score based on citrulline more specific and sensitive than either one based on signs and symptoms (such as World Health Organization and the National Cancer Institute-Common Terminology Criteria for Adverse Event (NCI-CTCAE) scales) or on sugar permeability tests.

In chapter 5 the use of plasma citrulline was compared to albumin concentrations which clinicians often rely on to indicate, among other things, intestinal function. The course of citrulline and albumin was evaluated in a cohort of 106 patients treated with different conditioning regimens (myeloablative and non-myeloablative). It was shown that albumin levels were greatly influenced by inflammation, confirming it to be a “negative acute phase protein”. This was not the case for citrulline which proved to be a better marker of intestinal MBI.

In conclusion, citrulline has shown to be an objective, reproducible, specific and reliable marker for intestinal MBI in patients following a HSCT.

PART III: FEBRILE NEUTROPENIA OR FEBRILE MUCOSITIS?

In this part by measuring citrulline the role of intestinal MBI in the occurrence of fever and infections is studied.

In chapter 6 the role of intestinal MBI in the occurrence of bacteraemia was investigated. In a retrospective study of autologous HSCT recipients who had been given high-dose melphalan as conditioning regimen, we showed that bacteraemia only occurred in those patients with fever (defined as a temperature ≥ 38.5 °C) during neutropenia who had hypocitrullinaemia, i.e., citrulline below 10 μmol/L. This suggests that the severity of intestinal MBI determines whether or not bacteraemia occurs, rather than neutropenia per se. The extent of MBI determined the loss of the physical barrier thereby facilitating bacteraemia due to micro-organisms originating from the gut. All episodes of bacteraemia were either due to viridans streptococci and coagulase negative staphylococci which are known to originate from the gastrointestinal (GI) tract in these patients.

This was followed by a study involving a cohort of 163 patients who were treated with six different conditioning regimens for autologous and allogeneic HSCT. There was a striking pattern of inflammation (measured by CRP) following myeloablative conditioning with the onset of fever coinciding with occurrence of MBI. Furthermore the inflammatory response and fever corresponded to the occurrence of MBI whether or not bacteraemia developed suggesting that fever is the direct consequence of MBI alone, irrespective of the duration of neutropenia, at least for some HSCT recipients. Hence MBI-related fever might be better termed “febrile mucositis” instead of “febrile neutropenia”. Moreover, patients with fever who developed bacteraemia during neutropenia also had hypocitrullinaemia, reflecting the central role of MBI in the triad of MBI, inflammation and bacteraemia (chapter 7).
To further untangle the relationship between intestinal MBI, neutropenia and the occurrence of inflammation, fever and bacteraemia, we performed a study in which a non-myeloablative regimen was compared with a myeloablative regimen to prepare for a HSCT with blood cultures being obtained for screening at regular intervals from admission onwards as well as at the onset of fever to investigate bacteraemia. This study showed that myeloablative treatment induced severe intestinal MBI manifested by hypocitrullinaemia, which was accompanied with an inflammatory response and bacteraemia in 44% of cases. By contrast, treatment with a non-myeloablative regimen was accompanied by less MBI, only modest inflammation and no bacteraemia. However both groups experienced profound neutropenia and its duration was significantly longer for those receiving the non-myeloablative regimen. These results suggest that hypocitrullinaemia defines the period of risk for bacteraemia better than does neutropenia and that measuring citrulline may prove useful in deciding who needs antimicrobial treatment and when (chapter 8).

PART IV: CONSEQUENCES OF USING CITRULLINE IN CLINICAL PRACTICE

In this last part we investigated how we might use the knowledge gained from studying MBI and the kinetics of citrulline to improve the management of febrile episodes of patients who received intensive cytotoxic treatment for cancer.

The recombinant human keratinocyte growth factor palifermin was shown to reduce the MBI of the oral cavity after TBI-containing regimens to prepare for allogeneic HSCT but not after other regimens. A protective effect of palifermin on intestinal MBI was seen when measured by permeability tests on the intestinal barrier of HSCT recipients who had been given BEAM as conditioning therapy. Therefore we also performed a study in a cohort of HSCT recipients given BEAM but used citrulline as a marker for intestinal MBI. While our study failed to detect any clinical relevant impact of palifermin on intestinal MBI it illustrated the usefulness of measuring citrulline for evaluating new therapies and strategies directed at the preservation on enterocyte mass, intestinal integrity and function (chapter 9).

Because of the central role MBI plays, we hypothesized that the onset of hypocitrullinaemia might help identify the period when a patient is at highest risk of developing fever and bacteraemia. To study this we investigated the occurrence of inflammatory and infectious complications of a conditioning regimen that was started on different days in a homologous group of HSCT recipients. We showed that by planning the start of the conditioning we could limit the risk of patients developing fever and bacteraemia during the weekend. This is important for our usual care as it could make managing HSCT recipients outside the hospital feasible and safe if accompanied by regular outpatient visits (chapter 10).

Finally, to gain a better insight into the true cause of fever in the patient with cancer, we postulated the new paradigm “febrile mucositis” in a review that also summarized the existing evidence for “febrile neutropenia”. We showed that, at least for some HSCT recipients, fever
is a direct consequence of MBI alone and that neutropenia plays a minor role or none at all. The term “febrile neutropenia” is misleading for these patients, as it does not reflect the true nature of fever induced by cytotoxic therapy and we suggest that the term “febrile mucositis” may be more appropriate. These patients are unlikely to benefit from adding or changing antimicrobials empirically or from treatment with growth factors such as G-CSF but may be exposed unnecessarily to the risk of side effects of these agents. Even though patients with fever during neutropenia will still need to be treated initially with antimicrobial therapy given empirically, switching or adding antimicrobials on the basis of persistent unexplained fever (≥ 3 days of fever) or “FUO” should be discouraged. Instead, a vigorous attempt should be made to diagnose or exclude infection. This approach will reduce the prescription of antimicrobial therapy and G-CSF, thereby helping to reduce health care costs, antimicrobial resistance and the risk of creating an imbalance between harmful and protective bacteria. Furthermore, the paradigm “febrile mucositis” will help open new doors to further improve the supportive care of cancer patients (chapter 11).

**FUTURE PERSPECTIVES**

The following recommendations can be made for the role of citrulline in:

**A) POST-TRANSPLANT PERIOD OF HSCT RECIPIENTS**

Several studies over the last decades have shown that MBI is not only one of the most debilitating side-effects of the intensive treatment prior to a HSCT, but is also an important determinant in the occurrence of post-transplant complications. It is associated with inflammatory and infectious complications, occurrence of acute graft versus host of the gut, greater use of total parental nutrition and opioids analgesics, as well as increased costs, length of stay and mortality. Citrulline is a valuable biomarker of intestinal MBI in HSCT recipients and allows for a standardized assessment of MBI and can support the management of MBI-related complications. Thus the time seems ripe for an alternative approach in HSCT recipients in which MBI plays a more important role than it has before.

This can potentially result in the following benefits:

- Knowledge concerning the course of MBI can be used to limit antimicrobial therapy to those most at risk, which can limit the emergence of antimicrobial resistance, reduce the risk of inducing dysbiosis and help contain health care costs. As example, antibacterial prophylaxis may be most beneficial to patients who develop MBI; and in patients who are treated with empirical antibiotics because of fever, simply switching or adding antimicrobials for persisting “FUO” should be discouraged. Currently in the Netherlands a ’short versus extended treatment with a carbapenem for high-risk febrile neutropenia in hematology patients with “FUO” (SHORT trial)’ has been started to answer the question whether or
not it is safe to discontinue the empirical antibiotic therapy of patients with “FUO” after 3 days.⁵

- The workload of nurses can be reduced if the risk period for fever and infections is determined by MBI instead of neutropenia. The feasibility of managing at least some of HSCT recipients outside the hospital could also be investigated under strict defined circumstances.
- MBI is likely to be the main determinant for not only choosing nutritional support but also how it is given. Total parenteral nutrition (TPN) is still the only option when there is severe malfunction of the gut, GI-fistulae or prolonged ileus, but patients may benefit more from enteral support once intestinal reconstitution occurs.⁶ For instance, TPN is probably only necessary for a limited time after myeloablative regimens whereas patients given non-myeloablative regimens probably benefit more from enteral nutrition. If citrulline levels are able to guide the best approach for nutritional support this will most likely lead to a reduction of TPN use as well as thrombosis and catheter related infections. Studies are eagerly awaited in HSCT recipients to investigate this more thoroughly.
- MBI could play a role in determining the optimal route for administering medication, including antimicrobial agents.

Clearly, formal studies are necessary to determine the specific thresholds of citrulline levels at which certain actions should be taken or the course of actions changed and to evaluate whether actions based on the severity of MBI really do lead to the expected improvements in the management of HSCT recipients in terms of reduced morbidity and mortality, better quality of life and lower health care costs.

B) GASTRO-INTESTINAL ACUTE GRAFT-VERSUS-HOST DISEASE (GI-GvHD)

Citrulline may also have a role in measuring the intestinal damage associated with GI-GvHD and therefore in monitoring the response to treatment thereby allowing timely adjustments in case of failure. However the use of citrulline should be compared to other biomarkers, such as regenerating islet-derived-3-α (REG3α) and albumin.

- REG3α, a C-type lectin (an antimicrobial protein) secreted by Paneth cells, has been identified as a biomarker of GvHD of the lower GI tract that can differentiate GI-GvHD from non-GvHD diarrhoea.⁷ Moreover, REG3α levels were prognostic with regards to steroid responsiveness of GvHD and post-transplant mortality.⁸,⁹

- Albumin is another organ specific biomarker for GI-GvHD. In allogeneic HSCT recipients prepared with reduced intensity regimens, decreases in serum albumin before aGvHD onset have substantial predictive value for aGvHD severity and mortality.¹⁰ The albumin level is also an independent prognostic factor for acute GI-GvHD, in contrast with acute GvHD of the skin.¹¹ Albumin levels on day 5 after starting steroid treatment could be used as a predictor of GvHD response to steroid treatment.¹²
Such biomarkers can be used in the future to generate a grading system that will allow better risk stratification and rapid identification of those patients with severe GI damage in whom standard treatment is likely to be insufficient and who need pre-emptive intensification of aGvHD therapy.

C) SOLID TUMOURS
Beside HSCT recipients, also other patients treated with cytotoxic therapy for cancer can develop intestinal MBI. The exact role for MBI in the treatment of solid tumours is less clear but MBI may also contribute to fever and infection. Also severe MBI should lead to a reduction of the dose in subsequent treatment cycles to prevent potentially fatal complications. Investigations using citrulline are awaited especially for regimens that induce fever during neutropenia in more than 20% of the patients.

D) EXPLORING REMEDIES TO AMELIORATE MBI
We need to find ways of preventing or ameliorating MBI to be able to further intensify our treatment in order to cure more patients, since this is an important dose-limiting toxicity. For example, restoring mucosal health or at least preventing MBI could prevent inflammation, microbial translocation and bacteremia. Citrulline can be used to explore remedies to ameliorate MBI.

It may be fruitful to borrow insights and ideas from other medical areas such as inflammatory bowel disease and GvHD that are characterized by mucosal barrier damage and dysregulated innate immunity. Promising approaches, include the use of growth factors e.g. R-spondin-1, Interleukin-22 and glucagon-like peptide to stimulate intestinal stem cells to help restore the anatomical barrier, as well as modulation of pattern-recognition receptors and the gut microbiota.13-16 Another approach might be to exploit pathways that enable tissue repair and resolution of the inflammatory process, by using anti-inflammatory cytokines (such as interleukin 10), or cytokines inhibitors (such as IL-1Ra), or scavengers that neutralize the effects of pathogen-associated-(PAMPs) and danger-associated- molecular patterns (DAMPs) (like anti-HMGB-1), or by correcting deficiencies in antimicrobial peptides (e.g. lactoferrin and defensins).17-20 Success in finding ways to prevent or treat MBI will be an important advance in the care of those patients who still need treatment of their cancer with cytotoxic regimens that induce MBI.

REFERENCES


5. www.short-trial.nl


19. Carvalho FA, Aitken JD, Gewirtz AT, Vijay-Kumar M. TLRs activation induces secretory interleukin-1 receptor antagonist (sIL-1Ra) and reduces inflammasome-associated tissue damage. Mucosal Immunol 2011; 4: 102-111.

CHAPTER 13

NEDERLANDSE
SAMENVATTING
Voor veel patiënten met een hematologische maligniteit (bloedkanker) is het ondergaan van een stamceltransplantatie de enige mogelijkheid op genezing. Zo’n stamceltransplantatie traject omvat (1) een intensieve cytotoxische (celdodende) behandeling (bestaande uit chemotherapie met of zonder radiotherapie (bestraling)) die gericht is op het doden van snel delende kankercellen, gevolgd door (2) het teruggeven van lichaamseigen (autoloog) of lichaamsvreemde (allogene) stamcellen. Vanuit deze stamcellen worden in het beenmerg, waar de bloedaanmaak plaatsvindt, weer gezond bloedcellen gevormd.

Een van de belangrijkste complicaties van deze behandeling is het optreden van koorts en infecties. Koorts treedt bij ongeveer 4 op de 5 stamceltransplantatie patiënten op. In 1 op de 5 patiënten wordt daarbij een klinische infectie, zoals een longontsteking, gevonden en in een derde tot de helft van de patiënten is er een microbiologische infectie, zoals een bacteriëmie (dat wil zeggen, dat er bacteriën in het bloed aanwezig zijn). Patiënten kunnen hierdoor ernstig ziek zijn en ook overlijden. Voor 7% van de patiënten die een autologe stamceltransplantatie hebben ondergaan en voor 13-17% van de patiënten die een allogene stamceltransplantatie ondergaan, is een infectie de primaire oorzaak waaraan zij overlijden. De reden voor het optreden van koorts en infecties is voornamelijk dat cellen van het afweersysteem ook voor het overgrote deel snel delend zijn en ernstig beschadigd raken door de intensieve cytotoxische behandeling. Omdat patiënten door deze afgenomen afweer erg kwetsbaar zijn voor infecties, wordt er zodra een patiënt koorts ontwikkeld direct gestart met zeer breed dekkende antibiotica (veelal carbapenems of 3de/4e generatie cefalosporines). Blijft de koorts ook na 3-4 dagen aanwezig, zullen hieraan vaak nog andere antimicrobiële middelen worden toegevoegd. Nadien wordt gezien de kwetsbaarheid van de patiënt meestal relatief lang gewacht voordat de antimicrobiële therapie weer gestaakt wordt.

Dit betekent dat door de koorts en de infecties er een groot beroep gedaan wordt op de ondersteunende zorg, patiënten langer opgenomen blijven en kosten voor de zorg flink stijgen. Daarnaast zorgt het antibiotica gebruik voor ontregeling van de microbiële flora (ook wel het microbioom van de patiënt genoemd), worden patiënten blootgesteld aan de bijwerkingen van de antibiotica en zorgt uitgebreid en langdurig gebruik van antibiotica, voor het ontstaan van antibiotica resistentie.

De toenemende antibiotica resistentie en de alsmaar stijgende zorgkosten zijn belangrijke factoren die de behandeling van (hematologische) patiënten met kanker in de toekomst bedreigen.
HET MICROBIOOM EN DE AFWEER VAN DE PATIËNT

Ieder mens bestaat uit 10 miljoen kernhoudende cellen en ter hoogte van de oppervlakken van het lichaam, zowel aan de buiten- als de binnenzijde, leven er nog eens 10 x zoveel bacteriën, namelijk zo’n 100 miljoen (Figuur 1).

Figuur 1. Microbioom van de mens

Aantal micro-organismen die leven in onze darmen, op onze huid en andere lichaamsoppervlakken.


Dit lichaamseigen microbioom speelt een belangrijke rol in het functioneren van de mens en helpt, in geval van een intacte anatomische barrière (zoals huid of spijsverteringsstelsel) om het lichaam te beschermen tegen pathogene (ziekmakende) micro-organismen. Echter bij beschadiging van de anatomische barrière raakt deze interactie met micro-organismen ontregeld. Hierdoor kunnen micro-organismen die zich normaal gesproken onschuldig gedragen, opeens voor een infectie zorgen. Zodra bacteriën er in slagen om de barrière van bijvoorbeeld huid of darm te passeren, zullen specifieke witte bloedcellen, neutrofielen genoemd, gaan proberen de bacteriën onschadelijk te maken. Als neutrofielen hier onvoldoende in slagen, worden vervolgens andere soorten bloedcellen voor de afweer geactiveerd.
GEVOLGEN VAN DE CYTOTOXISCHE BEHANDELING OP DE AFWEER

1. NEUTROPENIE (VERLAAGD AANTAL NEUTROFIELEN)
Van oudsher is gedacht dat de belangrijkste oorzaak voor de afgenomen afweer bij stamceltransplantatie patiënten, in de eerste periode na de transplantatie, komt doordat de cytotoxische behandeling zorgt voor afname van neutrofieLEN. Het beenmerg waar bloedcellen worden gemaakt, is snel delend en raakt ernstig beschadigd door de cytotoxische behandeling. Daar neutrofieLEN een korte levensduur hebben, zal de schade aan het beenmerg hier het eerste zichtbaar worden. Koorts die optreedt bij neutropenie, noemt men “febriele neutropenie” en wordt gerelateerd aan de ernstige infecties die optreden. Op basis van neutropenie wordt bij stamceltransplantatie patiënten de risicoperiode bepaald waarbinnen infecties kunnen optreden en extra controles door verpleegkundig personeel nodig zijn. Op het moment van vaststellen van “febriele neutropenie” wordt direct met empirische (dat wil zeggen: op basis van ervaring) antibiotica gestart. Deze antibiotica worden op zijn minst 7 dagen gegeven en vaak pas gestaakt als de neutropene periode over is.

2. BESCHADIGING VAN DE ANATOMISCHE BARRIÈRE.
De anatomische barrière (zoals huid en spijsverteringstelsel) is echter ook snel delend en zal als gevolg van de cytotoxische behandeling eveneens beschadigen. Vooral de gevolgen ter hoogte van de darm zijn hierbij interessant, omdat hier 90% van de blootstelling aan micro-organismen plaatsvindt.
Vooral nog wordt in de zorg van stamceltransplantatie patiënten hieraan echter weinig aandacht besteed.

2.1 Mucositis
De schade die wordt aangericht aan de slijmvliezen van het spijsverteringskanaal wordt mucositis of mucosale barrière schade genoemd.
Vaak wordt mucositis onderverdeeld in orale mucositis (schade aan het mondslijmvlies) en intestinale mucositis (schade aan het darmslijmvlies).
Mucositis wordt over het algemeen gezien als een vervelende bijwerking van de gegeven cytotoxische behandeling, die echter ook spontaan weer overgaat. Doordat orale mucositis redelijk eenvoudig gezien kan worden door in de mond te kijken, is hier veel meer over bekend dan over intestinale mucositis.

2.1.1 Scoringsmethoden op basis van symptomaticologie
Orale mucositis is zichtbaar in de mondh holte als rood, snel bloedend slijmvlies al dan niet in combinatie met zweren. Het geeft pijnklachten waardoor patiënten minder gaan eten en drinken. De scoringsmethoden van o.a. de Wereldegezondheidsorganisatie (WHO) baseren zich op deze objectieve en functionele gegevens om orale mucositis in kaart te brengen.
Dit is echter lastig als het intestinale mucositis betreft. Intestinale mucositis kenmerkt zich door misselijkheid, braken, buikpijn en diarree waardoor de eetlust afneemt en gewichtsverlies optreedt. Deze symptomen kunnen echter ook directe bijwerkingen zijn van de gegeven cytotoxische behandeling of van andere toegediende medicatie. Bijvoorbeeld morfine gebruik, gegeven voor de pijn, kan constipatie geven en dus tekenen van intestinale mucositis vertroebelen. Bovendien kan intestinale mucositis alleen gezien worden wanneer met behulp van een endoscoop (een kijkbuis) in de darm gekeken wordt en er eventueel een darmbiopt (hapje slijmvlies) wordt afgenomen. Bij pathologisch onderzoek zal dan atrofie (afsterven) van de villi (darmvlokken) gezien worden. Endoscopisch onderzoek is echter ingrijpend en mogelijk ook gevaarlijk voor de patiënt vanwege het risico op infectie, bloedingen (omdat ook de bloedplaatjes laag zijn door de effecten op hetbeenmerg) en perforatie (ontstaan van een gaatje in de darm). In de praktijk wordt dit onderzoek daarom zeker niet routinematig toegepast.

2.1.2. **Absorptie- en permeabiliteitstesten**

Om toch meer te weten te komen over intestinale mucositis is daarom gekeken naar veranderingen in het functioneren van de darm zodra er schade optreedt. De beschadiging van de darm veroorzaakt veranderingen in de absorptie (opname) en permeabiliteit (doorlaatbaarheid) van verschillende stoffen. Om dit vast te leggen is gebruik gemaakt van verschillende suikers en soms ook radioactief gelabelde stoffen. Deze worden oraal (via de mond) ingenomen en later in de urine gemeten. De mate waarin bepaalde suikers al dan niet worden opgenomen via het darmslijmvlies om nadien vanuit de bloedbaan in de urine te worden uitgescheiden, zegt iets over de darmfunctie. Op deze manier wordt direct na toediening van een cytotoxische behandeling, die intestinale mucositis veroorzaakt, een afname van de darmfunctie gezien. In de dagen die hierop volgen, neemt de functie van de darm steeds verder af, totdat vaak na ongeveer 10-14 dagen de grootste afname in darmfunctie wordt bereikt. Dit dieptepunt wordt gevolgd door een geleidelijk herstel van de darmfunctie. Beperkingen bij deze absorptie- en permeabiliteitstesten zijn o.a. verschillen in maagledigingsnelheid, darmpassagietijd en nierfunctie. Bovendien zijn deze onderzoeken erg belastend voor de patiënt, wat in de praktijk betekent dat de patiënt dit onderzoek vaak zal weigeren juist op het moment dat de schade aan de darm het ergst zal zijn. Daarom is uitgekeken naar een andere manier om de schade aan de darm, als gevolg van de cytotoxische behandeling, vast te leggen. De voorkeur ging hierbij uit naar een marker die bepaald zou kunnen worden in het bloed. Dit, daar bij stamceltransplantatie patiënten, al omwille van allerlei andere redenen, vrijwel dagelijks bloed wordt afgenomen. Bovendien gebeurt deze bloedafname veelal via een centrale lijn, waardoor het weinig belastend is voor de patiënt.

2.1.3. **Citrulline**

In 2003 verscheen een artikel over patiënten waarbij darmvlokken afsterven o.a. als gevolg van coelieziek (glutenintolerantie). Hierin werd beschreven dat bij deze patiënten de mate van darmschade kon worden vastgesteld door het meten van een aminozuur in het bloed. Dit aminozuur is citrulline. Citrulline is een interessante marker om de mate van darmschade te meten, omdat
alleen het slijmvlies van de darm (om precies te zijn: de enterocyt) dit in aanzienlijke hoeveelheden afgeeft aan de bloedbaan. Vervolgens passeert citrulline de lever zonder dat dit een significante invloed heeft op de totale citrulline concentratie en wordt het enkel door de nieren opgenomen uit de bloedbaan en omgezet in een ander aminozuur namelijk arginine. Dit betekent dat de hoeveelheid citrulline bij een normale nierfunctie direct gerelateerd is aan het aantal functionerende enterocyten. Bij beschadiging van het darmepitheel zal het gehalte aan citrulline in het bloed dalen.

Citrulline is vervolgens ook onderzocht in andere categorieën van patiënten, onder meer bij patiënten met een kortedarmsyndroom (dit syndroom wordt gekenmerkt door een tekort aan goed functionerend darmweefsel) en patiënten die een darmtransplantatie hadden ondergaan. Ook bij deze patiënten bleek de citrulline concentratie in het bloed een goede weergave van het functionerende darmepitheel. Zodanig is in ons ziekenhuis ook onderzocht of citrulline een geschikte marker zou kunnen zijn voor de schade aan de darm, die wordt aangericht door de intensieve cytotoxische behandeling, bij stamceltransplantatie patiënten. Eerdere studies wekken inderdaad deze indruk.

In dit proefschrift wordt daarom nu de waarde van citrulline in deze patiëntengroep nader onderzocht. Allereerst wordt nagegaan in hoeverre citrulline een geschikte marker is voor intestinale mucositis bij alle stamceltransplantatie patiënten. Vervolgens wordt gebruik makend van citrulline bepalingen de rol van intestinal mucositis op het optreden van koorts en infecties onderzocht. De inzichten die hieruit voortvloeien, worden gebruikt om na te gaan in welke mate het bepalen van citrulline mogelijkheden biedt om de zorg voor de (hematologische) patiënt met kanker verder te verbeteren.

Dit proefschrift bestaat uit 4 delen.

DEEL I. DE HUIDIGE PRAKTIJK.
In dit eerste deel wordt beschreven waaruit onze huidige zorg ten aanzien van een infectie bij de neutropene patiënt met een hematologische kanker bestaat en hoe dit beleid in de loop der jaren tot stand is gekomen.

In de 20e eeuw is er namelijk veel veranderd met betrekking tot de zorg voor de (hematologische) patiënt met kanker. Zo is het in de eerste helft van de 20e eeuw vrijwel onmogelijk om te genezen en overlijden bijna alle patiënten aan de gevolgen van hun ziekte. Dit verandert in de 60er jaren met de komst van cytotoxische middelen. Hierdoor blijven sommige patiënten leven. Terwijl er ook andere patiënten zijn die juist door de complicaties van deze behandeling overlijden. Infectie vormt hierbij één van de belangrijkste doodsoorzaken.
In 1966 wordt voor het eerst beschreven dat deze ernstige infecties optreden tijdens, door cytotoxische behandeling geïnduceerde, neutropenie. Ontdekt wordt dat naarmate de ernst
en duur van de neutropenie toeneemt, er ook meer kans is op ernstige en levensbedreigende infecties. Kort daarop wordt ook ingezien dat er minder mensen aan een infectie tijdens neutropenie overlijden wanneer er al bij de eerste tekenen daarvan, dat wil zeggen bij het optreden van koorts, gestart wordt met antibiotica en niet gewacht wordt tot er uit de bloedkweken eindelijk een bacterie wordt gekweekt. Dit leidt tot de introductie van de term “febriele neutropenie” (koorts bij neutropenie). De empirische aanpak (aanpak op basis van ervaring), waarbij er bij “febriele neutropenie” direct met breed spectrum antibiotica wordt gestart, wordt de standaardzorg voor de patiënt met kanker.

In de jaren die volgen zijn er steeds meer patiënten die genezen van kanker. Enerzijds komt dit door verbeteringen in de ondersteunende zorg, waaronder de empirische aanpak en de ontwikkeling van steeds nieuwere en betere antibiotica. Anderzijds wordt dit bereikt door een steeds intensievere cytotoxische behandeling (die ook mogelijk wordt door de betere ondersteuning) en gaat stamceltransplantatie een onderdeel vormen van het behandelplan bij de patiënt met een hematologische kanker.

Door deze intensievere behandeling raakt de afweer echter op veel meer fronten tegelijk beschadigd. Het huidige beleid ten aanzien van koorts en infecties is echter nog steeds gericht op het concept van “febriele neutropenie” (hoofdstuk 2).

**DEEL II. INTRODUCTIE VAN CITRULLINE ALS BIOMARKER VAN INTESTINALE MUCOSITIS**

_Hier wordt citrulline geïntroduceerd als marker voor darmschade als gevolg van cytotoxische behandeling._

In _hoofdstuk 3_ wordt in een review artikel beschreven welke rol citrulline kan hebben als marker van darmschade bij stamceltransplantatie patiënten. Hierbij wordt citrulline als marker afgezet ten opzichte van de tot op heden toegepaste methoden. Tevens wordt geïllustreerd hoe door het meten van citrulline de zorg voor patiënten (wellicht) verbeterd kan worden, namelijk door gerichtere toepassing van medicatie, antibiotica, en nutritionele ondersteuning.

In _hoofdstuk 4_ wordt citrulline als marker van intestinale mucositis getest in 94 patiënten die een stamceltransplantatie ondergaan en vooraf behandeld worden met verschillende cytotoxische schema’s. Hierbij wordt toegelicht dat met behulp van citrulline het mogelijk is om te laten zien dat elk schema zijn eigen patroon van het optreden van intestinale mucositis (darmslijmvliesbeschadiging) heeft. Vervolgens worden twee scoringsmethoden beschreven, die allebei gebruik maken van de citrulline bepaling. De ene methode is meer geschikt voor de dagelijkse praktijk en baseert zich op de werkelijke hoogte van het citrulline gehalte in het bloed. Citrulline waarden onder de 10 μmol/L (hypocitrullinemie) zijn hierbij overeenkomend met een beeld van totale villusatrofie, dat wil zeggen de meest ernstige beschadiging van het darmslijmvlies. De andere methode baseert zich op het totale verloop van het citrulline gehalte.
gedurende 30 dagen en kan daardoor beter voor onderzoeksdoeleinden gebruikt worden om op deze manier verschillende behandelingsschemes of interventies met elkaar te vergelijken. Uit beide methoden wordt echter duidelijk dat bij stamceltransplantatie patiënten citrulline een zeer sensitieve en specifieke marker is om intestinale mucositis weer te geven.

Vervolgens wordt citrulline als marker van intestinale mucositis vergeleken met albumine. Dit, omdat veel clinici albumine gebruiken als een indicator voor de ernst van mucositis. Uit deze studie blijkt dat de waarde van albumine echter in belangrijke mate beïnvloed wordt door ontstekingsprocessen, terwijl dit niet voor citrulline geldt (hoofdstuk 5).

Geconcludeerd kan worden dat op basis van de sensitiviteit, specificiteit, de eenvoudige methodologie, en de relatief lage kosten citrulline de eerste keuze zou moeten zijn voor het objectief meten en monitoren van de door cytotoxische behandeling geïnduceerde intestinale mucositis.

**DEEL III: FEBRIELE NEUTROPENIE OF FEBRIELE MUCOSITIS?**

*In dit derde deel wordt gekeken naar de rol die intestinale mucositis speelt in het optreden van koorts en infecties bij patiënten die een stamceltransplantatie ondergaan.*

Figuur 2. Optreden van neutropenie en mucositis in relatie tot koorts en infecties

Deze figuur toont het verloop van de temperatuur, het optreden van infecties, mucositis en neutropenie nadat een stamceltransplantatie patiënt gestart is met chemotherapie. In het bovenste deel staat het verloop van temperatuur en het optreden van infecties. Het middelste deel toont wanneer mucositis optreedt. Het onderste deel laat het neutrofielen aantal per dag zien vanaf het moment dat er gestart is met chemotherapie.
Zoals in figuur 2 wordt getoond treden koorts en infecties op ten tijde van zowel de neutropenie als mucositis.
Met behulp van citrulline is vervolgens uitgezocht wat het belang van intestinale mucositis bij het optreden van deze inflammatoire en infectieuze complicaties is.

In hoofdstuk 6 wordt nagegaan wat de rol van intestinale mucositis is bij het ontstaan van een bacteriëmie. Dit wordt onderzocht in een uniforme groep van patiënten, die allen behandeld zijn voor een multipel myeloom, met hoge dosis melfalan gevolgd door een autologe stemceltransplantatie. In deze groep wordt gekeken naar de patiënten die “neutropene koorts” ontwikkelen, waarbij er wel of niet sprake is van een bacteriëmie. De citrullinewaarden van patiënten met een bacteriëmie worden vervolgens vergeleken met de citrullinewaarden van patiënten met enkel koorts. Deze studie toont aan dat de patiënten met een bacteriëmie significant meer darmschade hebben op het moment van koorts; alle patiënten met een bacteriëmie hebben een citrulline onder de 10 µmol/L. Tevens worden alle bacteriëmiën veroorzaakt door ofwel een coagulase negatieve stafylokok ofwel een streptococcus viridans. Eerdere studies hadden al laten zien dat deze typen bacteriën uit het spijsverteringskanaal afkomstig kunnen zijn. Dit suggereert dat de ernst van de schade aan de darm bepaalt of er een bacteriëmie optreedt en niet de neutropenie.

Dit wordt gevolgd door een studie waarin 163 stemceltransplantatie patiënten hebben deelgenomen, die met 6 verschillende cytotoxische schema’s zijn behandeld, gevolgd door ofwel een autologe ofwel een allogene stemceltransplantatie. In deze studie is gekeken naar de rol die intestinale mucositis speelt bij het optreden van koorts en inflammatie (gemeten door CRP). Door de onderlinge verschillen in het ontstaan van intestinale mucositis tussen de behandelingsschema’s wordt zichtbaar dat het optreden van ernstige mucositis de belangrijkste factor is in het ontstaan van koorts bij patiënten, die behandeld zijn met schema’s die ernstige mucositis induceren (myeloablatieve schema’s). Het optreden van koorts en inflammatie correspondeert met het ontstaan van ernstige intestinale mucositis, ongeacht of er wel of geen sprake is van bacteriëmie. Deze studie veronderstelt dat in een aantal patiënten het ontstaan van koorts louter het gevolg is van intestinale mucositis en dat de duur van de neutropenie hierin geen rol bij speelt. Bij deze patiënten kan men daarom beter spreken van “febriele mucositis” (koorts bij mucositis) in plaats van “febriele neutropenie” (neutropene koorts). Overigens hebben ook in deze studie alle patiënten met een bacteriëmie een citrulline onder de 10 µmol/L. Intestinale mucositis lijkt daarom een centrale rol te spelen in zowel het optreden van koorts als in het ontstaan van een bacteriëmie (hoofdstuk 7).

Om de relatie tussen intestinale mucositis, neutropenie en het optreden van koorts en bacteriëmie verder te ontrafelen, wordt vervolgens een studie uitgevoerd, waarbij stemceltransplantatie patiënten die behandeld zijn met een schema dat veel mucositis geeft (een
Zogenaamd myeloablatief schema), worden vergeleken met stamceltransplantatie patiënten die behandeld zijn met een schema dat nauwelijks mucositis maar wel diepe en langdurige neutropenie geeft (een non-myeloablatief schema). In deze studie worden frequenter bloedkweken dan gewoonlijk afgenomen, namelijk minimaal 3x/week en ook zonder dat er sprake is van koorts. Deze studie laat zien dat ondanks de significant langere diepe neutropenie duur in het non-myeloablatieve schema, er significant minder koorts en bacteriëmieën optreden ten opzichte van het myeloablatieve behandeling.

Kortom, de belangrijkste factor in het ontstaan van koorts en bacteriëmieën bij patiënten die een stamceltransplantatie ondergaan is de ernst van de mucositis en niet de neutropenie (hoofdstuk 8).

DEEL IV: KLINISCHE CONSEQUENTIES

In dit laatste deel wordt gekeken naar mogelijke consequenties die voortvloeien uit de nieuw verworven kennis ten aanzien van de rol van intestinale mucositis, die gemeten kan worden middels citrulline in het bloed.

Zo wordt met behulp van citrulline bepalingen bij patiënten met een lymfoom (lymfeklierkanker) het beschermende effect van palifermine, een humane keratinocyten groeifactor, op het optreden van intestinale mucositis getest. Alle patiënten in deze studie zijn behandeld met het cytotoxiche schema BEAM (waarna een autologe stamceltransplantatie volgde), omdat permeabiliteitstesten bij dit schema reeds eerder dit beschermende effect van palifermine hadden getoond. Helaas kon dit beschermende effect van palifermine op het darmsluimvlies niet geobjecteerd worden door gebruik te maken van citrulline bepalingen. Wel toont deze studie het belang van citrulline aan in de beoordeling van de effectiviteit van een interventie voor intestinale mucositis (hoofdstuk 9).

Gebruik makend van eerdere observaties dat 1) het behandelingsschema het optreden van intestinale mucositis bepaald en dat 2) intestinale mucositis de belangrijkste factor in het ontstaan en optreden van koorts en bacteriëmieën is, wordt vervolgens onderzocht of door het beter plannen van een stamceltransplantatie het risico op inflammatoire en infectieuze problemen in het weekend beperkt kan worden. Dit is van belang omdat dit wellicht mogelijkheden biedt om, na de infusie van autologe stamcellen, (een deel van) het stamceltransplantatietraject op een veilige manier met frequente poliklinische controles buiten het ziekenhuis te laten verlopen. Hiervoor wordt retrospectief gekeken naar patiënten met een multipel myeloom die behandeld zijn met hoge dosis melfalan voorafgaande aan een autologe stamceltransplantatie. De melfalan is bij deze patiënten op verschillende dagen in de week gestart. Deze studie laat zien dat, op grond van het verwachte beloop van intestinale mucositis, de dag waarop de chemotherapie start van invloed is op het al dan niet optreden van infecties in het weekend (hoofdstuk 10).
Tot slot wordt, in een review artikel, het concept “febriele mucositis” geïntroduceerd, waarmee wordt weergegeven dat koorts als gevolg van mucositis kan optreden. Deze koorts is het gevolg van het ontstekingsproces geïnduceerd in het slijmvlies door de cytotoxische therapie. Omdat het om een ontsteking gaat en niet om een infectie is het toedienen van antibiotica minder logisch. Het doel van de introductie van de term “febriele mucositis” is om dokters bewust te laten worden van de invloed van mucositis op het ontstaan van koorts, waardoor er kritischer met antibiotica kan worden omgegaan. Dit is van belang omdat minder en dus ook korte antibiotica gebruik zal leiden tot minder ontregeling van het microbioom, minder ontwikkeling van antibiotica resistentie en meest waarschijnlijk ook een kortere opnameduur; dit zal uiteindelijk ook de kosten van de zorg voor de stamceltransplantatie patiënt doen afnemen. Nader onderzoek is echter nodig om de klinische implicaties die uit deze nieuwe inzichten kunnen voortvloeien, verder te bestuderen. Ook dient meer geld en mankracht beschikbaar te komen voor studies naar interventies met middelen, die een beschermend effect op de darmmucosa kunnen hebben. Immers, indien het lukt om het optreden van mucositis te beperken of te voorkomen zal dit een aanzienlijke verbetering betekenen in de zorg voor de patiënt met kanker (hoofdstuk 11).
APPENDICES
LIST OF ABBREVIATIONS
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>aGvHD</td>
<td>acute graft-versus-host disease</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphatic leukemia</td>
</tr>
<tr>
<td>ALI</td>
<td>acute lung injury</td>
</tr>
<tr>
<td>AML</td>
<td>acute myeloid leukemia</td>
</tr>
<tr>
<td>AMP</td>
<td>antimicrobial peptide</td>
</tr>
<tr>
<td>ARG</td>
<td>arginine</td>
</tr>
<tr>
<td>ARGase</td>
<td>arginase</td>
</tr>
<tr>
<td>ASL</td>
<td>argininosuccinate lyase</td>
</tr>
<tr>
<td>ASS</td>
<td>argininosuccinate synthetase</td>
</tr>
<tr>
<td>ATG</td>
<td>anti-thymocyte globulin</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BEAM</td>
<td>carmustine, etoposide, cytarabine and melphalan</td>
</tr>
<tr>
<td>Bu</td>
<td>busulfan</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphatic leukemia</td>
</tr>
<tr>
<td>CML</td>
<td>chronic myeloid leukemia</td>
</tr>
<tr>
<td>CoNS</td>
<td>coagulase-negative staphylococci</td>
</tr>
<tr>
<td>CRP</td>
<td>c-reactive protein</td>
</tr>
<tr>
<td>CT-scan</td>
<td>computer-assisted tomography-scan</td>
</tr>
<tr>
<td>CTCAE</td>
<td>common terminology criteria for adverse event</td>
</tr>
<tr>
<td>CVC</td>
<td>central venous catheter</td>
</tr>
<tr>
<td>Cyclo</td>
<td>cyclophosphamide</td>
</tr>
<tr>
<td>DAMP</td>
<td>danger-associated molecular pattern</td>
</tr>
<tr>
<td>DGS</td>
<td>daily gut score</td>
</tr>
<tr>
<td>DMS</td>
<td>daily oral mucositis score</td>
</tr>
<tr>
<td>G-CSF</td>
<td>granulocyte-colony stimulating factor</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
</tr>
<tr>
<td>Glu</td>
<td>glutamate</td>
</tr>
<tr>
<td>GLN</td>
<td>glutamine</td>
</tr>
<tr>
<td>GLN-ase</td>
<td>glutaminase</td>
</tr>
<tr>
<td>Gy</td>
<td>gray</td>
</tr>
<tr>
<td>HDM</td>
<td>high dose melphalan</td>
</tr>
<tr>
<td>HMGB-1</td>
<td>high mobility group box 1</td>
</tr>
<tr>
<td>HSCT</td>
<td>hematopoietic stem cell transplantation</td>
</tr>
<tr>
<td>HSP</td>
<td>heat shock protein</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ida</td>
<td>idarubicin</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IFNγ</td>
<td>interferon gamma</td>
</tr>
<tr>
<td>ISOO</td>
<td>International Society for Oral Oncology</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NFkB</td>
<td>nuclear factor-kappaB</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NMA regimen</td>
<td>non-myeloablative regimen</td>
</tr>
<tr>
<td>MA regimen</td>
<td>myeloablative regimen</td>
</tr>
<tr>
<td>MASCC</td>
<td>Multinational Association of Supportive Care in Cancer</td>
</tr>
<tr>
<td>MBI</td>
<td>mucosal barrier injury</td>
</tr>
<tr>
<td>MDS</td>
<td>myelodysplastic syndrome</td>
</tr>
<tr>
<td>MM</td>
<td>multiple myeloma</td>
</tr>
<tr>
<td>MPD</td>
<td>myeloproliferative disease</td>
</tr>
<tr>
<td>MUD</td>
<td>matched unrelated donor</td>
</tr>
<tr>
<td>OAT</td>
<td>ornithine aminotransferase</td>
</tr>
<tr>
<td>OCT</td>
<td>ornithine carbamoyl transferase</td>
</tr>
<tr>
<td>OM</td>
<td>oral mucositis</td>
</tr>
<tr>
<td>OMAS</td>
<td>oral mucositis assessment scale</td>
</tr>
<tr>
<td>ORN</td>
<td>ornithine</td>
</tr>
<tr>
<td>OVS</td>
<td>oral viridans streptococci</td>
</tr>
<tr>
<td>PAMP</td>
<td>pathogen-associated molecular pattern</td>
</tr>
<tr>
<td>PRR</td>
<td>pattern-recognition receptors</td>
</tr>
<tr>
<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>TBI</td>
<td>total body irradiation</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumor necrosis factor-alfa</td>
</tr>
</tbody>
</table>
DANKWOORD
DANKWOORD

Ik heb dit promotietraject met zeer veel plezier doorlopen en dat is in belangrijke mate te danken aan alle direct en indirect betrokkenen, die ik graag allen hierbij hartelijk wil danken (ook al kan ik niet iedereen speciaal benoemen).

PATIËNTEN
De onderzoeken die beschreven zijn, hadden nooit plaats kunnen vinden indien niet vele patiënten bereid waren geweest om hier aan mee te werken. Het was dan ook erg bijzonder om mee te mogen maken dat mensen die zelf al zo ziek zijn, veelal bereid zijn om (vooral door een extra bloedafname) te helpen de zorg in de toekomst nog beter te maken. Enkele van deze patiënten maken deel uit van mijn huidige praktijk in het Jeroen Bosch Ziekenhuis. Daarnaast zijn er ook velen patiënten uit deze praktijk geweest die steeds belangstellend informeerden naar mijn activiteiten in dit promotietraject. Hieruit heb ik veel energie geput en ben ik een ieder erg dankbaar voor.

COLLEGA’S

*Radboud universitair medisch centrum (Radboudumc)*

Op de dinsdagen dat ik op het Radboudumc ben, kom ik altijd “in een warm bad” terecht. Ik heb een kamer waar mijn naam opstaat (iets wat mij in de nieuwe locatie van het Jeroen Bosch Ziekenhuis niet gegeven is) en voel mij altijd welkom. Dit gevoel komt voort uit de inspanningen van velen: het secretariaat, verpleegafdeling E00, het laboratorium hematologie, het datacentrum, wetenschappelijk onderzoekers, arts-assistenten interne, fellows hematologie en alle stafleden van de hematologie. Het heeft er mede toe bijgedragen dat ik het promotietraject heb afgerond.

Omdat een aantal personen een belangrijke rol hebben vervuld in dit proefschrift, wil ik deze hieronder speciaal nog noemen:

Mijn promotor Nicole Blijlevens heeft met haar enthousiasme en gedrevenheid ervoor gezorgd dat ik aan dit promotietraject ben begonnen. Samen met haar zijn alle onderzoeken in dit boekje bedacht. Ondanks al mijn perifere ambities heeft zij gedurende het gehele traject altijd naar mij toe aangegeven “het volste vertrouwen te hebben in de goede afloop ervan”. Dit heb ik enorm in haar gewaardeerd.

Peter Donnelly is als mijn copromotor altijd de meest stabiele persoon in de begeleiding geweest. Hij gaf goede tips en aanwijzingen en liet mij vervolgens vrij in hoe ik het wilde gaan aanpakken. Over de laatste jaren ben ik mij steeds meer gaan realiseren wat een enorm geluk ik heb met zo’n ervaren copromotor. Het komt dan ook recht uit mijn hart als ik zeg: “It has been an honor and pleasure to work with him.”

Walter van der Velden is in 2013 copromotor geworden, maar was al veel eerder betrokken bij de onderzoeken in dit proefschrift. Hij is inhoudelijk kritisch en ziet de dingen scherp. Ik heb dit erg gewaardeerd.
Ton Feuth was t/m 2012 bij alle onderzoeken als statisticus betrokken, nadien heeft Ton de Haan dit van hem overgenomen. Met beiden had ik een prettige samenwerking en ze hebben mij erg geholpen bij alle statistiek.

Theo de Witte is bij aanvang van het promotietraject mijn promotor geweest. Hij heeft mij ondersteund in mijn plannen om een deel van mijn opleiding tot hematoloog in een perifeer ziekenhuis te doen, in combinatie met academisch onderzoekswerk. Uiteindelijk is dit voor mij een perfecte combinatie gebleken.

Met Ben de Pauw is het tweede hoofdstuk geschreven. Het is in een Amerikaans (leer)boek verschenen en zorgt er voor dat er op internationale congressen meermaals aan mij is gevraagd of ik misschien de auteur van dat hoofdstuk was. Ik ben er dan ook erg trots op en Ben dankbaar dat hij mij als fellow hematologie hiervoor destijds heeft benaderd.

Lenneke van Groningen, Roger Brüggemann, Michiel Schaap en Mihai Netea wil ik graag hartelijk danken voor hun bijdrage aan respectievelijk hoofdstuk 3, 5, 7 en 11.

Jeroen Bosch Ziekenhuis
Vanuit het Jeroen Bosch Ziekenhuis is er vanuit verschillende geledener, maar vooral het secretariaat, MOC, afdeling CSzuid, arts-assistenten interne en alle leden van de vakgroep interne/ MDL altijd veel interesse geweest voor mijn onderzoek. Ook het feit dat het woord mucositis regelmatig het ochtendrapport passeert, doet mij veel deugd.

FAMILIE EN VRIENDEN
Een prettige thuissituatie is van groot belang voor alle energie die je aan je werk kan besteden. Ik prijs mij dan ook erg gelukkig met een fijne familie en vriendenclub. Mijn 2 paranimfen zijn mijn zusje Krysta Herbers en mijn beste vriendin Carolina Laumans. Ik ben erg trots dat zij op de dag van de promotie naast mij staan. Ook mijn ouders en schoonouders kunnen allen aanwezig zijn, iets waar ik heel erg blij om ben. Mijn ouders hebben mij altijd gestimuleerd om mijn dromen waar te maken en bij de totstandkoming van dit proefschrift heeft mijn vader geholpen door allerlei gegevens in te voeren, ik ben hen beiden daar erg dankbaar voor. Mijn lieve Rob heeft mij alle ruimte gegeven om dit proefschrift af te ronden en nu het bijna zo ver is... heb ik weer allerlei ideeën om deze ruimte weer in te vullen (werk en niet-werk gerelateerd)!

Hartelijke groeten en tot de 21e augustus!

Alexandra
BIOGRAFIE & PUBLICATIELIJST
BIOGRAFIE


In hetzelfde jaar startte zij met haar promotieonderzoek bij Prof. dr. T. J. M. de Witte, Dr. N. M. A. Blijlevens en Dr. J. P. Donnelly. Na het afscheid begin 2013 van Prof. dr. T. J. M. de Witte als hoogleraar hematologie van het Radboudumc, werd N. M. A. Blijlevens benoemd tot hoogleraar en Alexandra’s promotor en werd Dr. W. J. F. M. van der Velden toegevoegd als copromotor. De werkzaamheden in dit promotietraject hebben geleid tot het proefschrift, zoals dit nu voor u ligt.

PUBLICATIES IN DE PERIODE VAN DIT PROMOTIETRAJECT


Hematopoietic stem cell transplantation (HSCT) provides effective treatment of hematological malignancies and other disorders. However, the procedure temporarily compromises the immune system resulting in damage to the gastrointestinal (GI) tract, called mucosal barrier injury (MBI), and neutropenia. The GI tract is host to billions of micro-organisms that not only share our body space but are essential for health. These micro-organisms constitute the microbiome and seldom cause us harm. However infection can, and does, occur when the mucosal barrier of the gut is injured. Fortunately antimicrobial agents are employed to prevent and treat infectious complications.

Never the less, as this thesis shows, almost all HSCT recipients develop fever that is the result of inflammation induced by certain drugs employed to prepare for the transplant. Furthermore, measuring the blood concentrations of the amino acid citrulline provides a means of assessing MBI and indicates that MBI, rather than neutropenia, defines the period of risk of fever and bacteraemia following transplant. With the ready availability of blood, this simple and reliable test can help to explore ways of ameliorating MBI to reduce inflammation and fever which, in turn, would lead to a more tailored approach to antibiotic treatment. This can only be a good thing as it will help reduce the risk of antimicrobial resistance.

Knowing the citrulline level could also help decide whether a patient needs to be admitted to hospital or can be treated safely at home.