Diploma Thesis

Impact of linezolid on hematopoietic engraftment in patients after umbilical cord blood stem cell transplantation

eingereicht von

Tanja Vilits

Zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Institut / Klinik für Hämatologie

unter der Anleitung von

ao. Univ. Prof. Dr. Albert Wölfler

Mag.rer.nat. Dr.techn. Franz Quehenberger

Graz, Dezember 2014

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Graz, Dezember 2014 *Tanja Vilits* eh

Danksagungen

Ich möchte an dieser Stelle die Gelegenheit nutzen und mich bei allen bedanken, die mich bei der Verfassung meiner Diplomarbeit unterstützt haben.

Zu Beginn möchte ich mich bei ao. Univ. Prof. Dr. Albert Wölfler bedanken, der mir die Möglichkeit gegeben hat dieses Thema zu bearbeiten und mich bei der Erstellung meiner Diplomarbeit kompetent und umfassend betreut hat.
Ebenso will ich einen herzlichen Dank an Mag.rer.nat. Dr.techn. Franz Quehenberger aussprechen, für das Korrekturlesen und die statistische Betreuung.

Meiner Familie danke ich für Ihre aufmunternden und motivierenden Worte, die ich während des Verfassens meiner Diplomarbeit benötigte. Meinem Papa für das Bereitstellen eines ‚Anti-Koller Zimmers', herrlichen Kaffee und aufbauenden Gesprächen.

Ich danke meinem Freund Leo, der mich während der gesamten Zeit unterstützt hat, mit mir durch Höhen und Tiefen gegangen ist und unendliche Geduld bewiesen hat.

Zusammenfassung

Hintergrund: Linezolid ist ein relativ neues Antibiotikum, welches für Infektionen, ausgelöst durch resistente Gram- positive Bakterien, zugelassen ist. Obwohl es eine gute klinische Verträglichkeit aufweist, wurde es mit einer hämatologischen Toxizität in der Allgemeinbevölkerung assoziiert. Daher könnte seine Verwendung im Rahmen einer allogenen Stammzellentransplantation zu einer möglichen Verzögerung des hämatologischen Engraftments führen. Insbesonders bei Transplantationen mit hämatopoietischen Stammzellen aus dem Nabelschnurblut könnte aufgrund der geringen Stammzelldosis ein adverser Effekt von Linezolid auf das hämatologische Engraftment auftreten.

Methodik: Die Daten von 35 PatientInnen, welche sich einer Transplantation von Nabelschnurblut- Stammzellen unterzogen haben, wurden retrospektiv analysiert. Das Ziel dieser Studie war, den Einfluss von Linezolid auf das neutrophile und thrombozytäre Engraftment zu bestimmen und weiters das Auftreten von Linezolid resistenten Keimen zu dokumentieren. Um die Daten zu analysieren wurden neben deskriptiver Statistik Kaplan Meier Kurven und Cox Regressionsmodelle herangezogen.

Ergebnisse: In dieser retrospektiven Studie wurden 35 PatientInnen (12 Frauen, 23 Männer) analysiert, die aufgrund einer malignen hämatologischen Grunderkrankung im Zeitraum von Dezember 2001 bis März 2013 mit einer Transplantation mit Nabelschnurblut-Stammzellen behandelt wurden. 28 PatientInnen (80%) wurden während der Phase der Neutropenie mit Linezolid behandelt, 7 PatientInnen (20%) wurden mit einem alternativen Antibiotikum behandelt. Im Median erfolgte die Linezolid-Behandlung für 16 Tage (Range 3-75 Tage). Das neutrophile und thrombozytäre Engraftment zwischen den beiden Gruppen war ähnlich und zeigte sowohl was die Dauer des Engraftments als auch das Erreichen bestimmter Zielwerte betraf, keine statistisch signifikanten Unterschiede. Linezolid-resistente Enterokokken wurden in Routinehautabstrichen von 3 Patienten, die mit Linezolid behandelt wurden, gefunden.

Schlussfolgerung: Im Rahmen dieser retrospektiven Untersuchung konnte gezeigt werden, dass Linezolid bei PatientInnen, die sich einer Transplantationen mit Nabelschnurblut-Stammzellen unterzogen haben, keinen negativen Einfluss auf das Engraftment von neutrophilen Granulozyten und Thrombozyten hat. Linezolid kann daher im Rahmen einer Nabelschnurbluttransplantation sicher verabreicht werden. Jedoch konnte in einem Teil der PatientInnen (10,7%) ein Auftreten von Linezolid-resistenten Enterokokken beobachtet werden.

Abstract

Background: Linezolid is approved for the treatment of infections caused by otherwise resistant Gram-positive bacteria, but has been associated with hematologic toxicity in the general population. Thus, there are concerns about its potential myelotoxicity in the setting of allogeneic stem cell transplantation (SCT). Umbilical cord blood SCT is a curative option in patients, who lack a suitable HLA-identical stem cell donor, but it associated with delayed and sometimes incomplete hematological recovery.

Methods: We retrospectively analysed the data of 35 patients having undergone umbilical cord blood SCT. The aim of our study was to evaluate the impact of linezolid treatment on neutrophil and platelet engraftment and the occurrence of linezolid resistant bacteria. We used descriptive statistical methods as well as Kaplan Meier curves and Cox regression models to analyse the data.

Results: We analysed 35 patients (12 female, 23 male) with underlying malignant haematological diseases treated with umbilical cord blood SCT. 28 patients (80%) were treated with linezolid during the neutropenic phase and 7 patients (20%) were treated alternatively. The median number of days on linezolid therapy after UCB transplantation was 16 days (range 3- 57 days). Neutrophil and platelet engraftment were similar between both groups and did not differ in the time to engraftment as well as in the percentages of patients achieving predefined neutrophil and platelet counts. Linezolid-resistant enterococci were identified in routine cutaneous swabs of 3 out of 28 patients.

Conclusion: We found that linezolid had no negative impact on neutrophil and platelet engraftment in patients having undergone UCB-HSCT. Hence it is suggested that linezolid can be administered safely in such patients. However, the occurrence of linezolid resistant gram- positive enterococci may be a matter of concern.

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Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ATG	Anti thymocyte globulin
CD	Cluster of differentiaton
CD34	Cluster of differentiation marker 34
CD4	Cluster of differentiation marker 4
CI	Confidence interval
CMV	Cytomegalovirus
CsA	Cyclosporin A
cSSSI	Complicated skin and skin structure infections
CVL	Central venous line
FDA	Food and drug administration
dUCB	Double Unit umbilical cord blood
E. faecium	Enterococcus faecium
ESBLE	Extended-spectrum-betalactamase-producing Enterobacteriaceae
FB2A2	Fludarabine+busulfan+antithymocyte globulin
fTBI	Fractioned total body irradiation
GCSF	Granulocyte colony stimulating factor
GvHD	Graft versus host disease
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cells

HSCT	Hematopoietic stem cell transplantation
LRVREF	Linezolid- and vancomycin-resistant Enterococcus faecium
MAO	Monoamine oxidases
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MM	Multiple Myeloma
MMF	Mycophenolate mofetil
MRSA	Methicillin- resistant Staphylococcus aureus
MSKCC	Memorial Sloan Kettering Cancer Center
MTX	Methothrexate
NHL	Non Hodgkin lymphoma
PBSC	Peripheral blood stem cells
PLT	Platelets
rRNA	Ribosomal ribonucleic acid
TCF	Fludarabine+cyclophosphamide+low dose total body irradiation
TGC	Tigecycline
TRM	Transplantation related mortality
UCB	Umbilical cord blood
VRE	Vancomycin resistant enterococcus

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1 Introduction

The concept of hematopoietic stem cell transplantation (HSCT) was developed more than 50 years ago. Originally intended to protect people from irradiation damage, it soon became clear that the infusion of bone marrow could have a therapeutic effect on malignancies as well. In 1959 E.D Thomas conducted the first human bone marrow transplantation in two patients with acute leukemia. He and his colleagues used whole body irradiation followed by infusion of the patients' identical twins bone marrow. They could prove engraftment of donor cells and a remission lasting for 12 weeks in one patient. (1,2)

Since then the number of patients undergoing HSCT has been continuously rising. Its highly curative potential in hematologic diseases has made it an often-used therapy and saved many patients' lives. For example, in the year 2013 452 patients underwent hematopoietic stem cell transplantation in Austria.
New sources of stem cells have evolved in the last decade: besides bone marrow, also stem cells collected from peripheral blood and umbilical cord blood can be used for transplantation. While transplantation of autologous stem cells is used in patients with multiple myeloma and relapsed lymphoma – both diseases spare hematopoietic stem cells – transplantation of allogeneic stem cells is performed in patients with acute leukemia. In these cases the patient´s hematopoiesis is completely replaced by HSC derived from a suitable allogeneic donor.

In this thesis I will focus on umbilical cord blood (UCB) transplantation, which has become increasingly common since the first successful transplantation in 1989. Over some advantages like immediate availability, no risk for the donor, less stringent HLA matching and lower incidence of graft-versus-host-disease (GvHD), slower hematopoietic recovery and opportunistic infections remain a problem due to low numbers of HSC infused with UCB. In this retrospective analysis I will evaluate the effect of a relatively novel antibiotic drug, linezolid, on hematopoietic recovery in patients undergoing UCB-HSCT. Linezolid has strong activity against (resistant) Gram-positive bacteria and is therefore often used in patients undergoing HSCT, but has been reported to negatively affect hematopoiesis in some patients. (8,9)

1.1 General definition of stem cell transplantation

Hematopoietic stem cells (HSC) are undifferentiated cells located in the human bone marrow, which are capable to replenish all types of blood cells. Since most blood cells, such as erythrocytes, leukocytes including granulocytes, lymphocytes and monocytes, and platelets are rather short lived, they need to be replenished a lifelong by HSC. As with all types of stem cells, their unique function in the human organism is the ability to reduplicate identical cells through mitosis for an indefinite period, with one daughter cell to differentiate and produce specialised effector cells. Because of this potential stem cells are able to 'replenish mature cell populations of the given tissue or organ, and to respond to stress by repairing damaged tissue.' (4) In general, embryonic and adult stem cells are distinguished. While pluripotent embryonic stem cells can produce all types of human tissues, adult stem cells produce newly differentiated cells throughout lifetime for one (unipotent) or more (pluripotent) organs. (1,10)
Research over the last three decades on stem cells has established specific markers for the identification of HSC. Human HSC are enriched within cells of the bone marrow expressing the marker CD34 on their surface. Therefore, flowcytometry analysis of blood and bone marrow cells detecting CD34 expression allows identification and estimation of HSC numbers, which is an absolute prerequisite for the application of HSC transplantation to patients in clinical practice.

The procedure of HSCT involves chemotherapy or irradiation (conditioning therapy) given to eradicate the patients' disease followed by the infusion of HSC. In the case of autologous stem cell transplantation the patient´s HSC are isolated prior to the conditioning procedure. After myeloablation with chemotherapy and/or irradiation the collected HSC are then re- infused to rescue hematopoiesis. In contrast, allogeneic stem cell transplantation refers to the infusion of HSC from a healthy donor into the patient (recipient). Allogeneic HSC donors must have a tissue (HLA) type that matches the recipient (for details see chapter 1.4.2). In contrast to autologous stem cell transplantation, allogeneic HSCT affects the recipient´s disease in two ways: In the first place, the applied conditioning regimen aims for eradication of (most of) the malignant cells. Secondly the donor´s T- cells,

which are infused along with the HSC are able to recognise antigens of the recipient´s leukemia or tumor cells leading to a graft-versus-leukemia/tumor effect. This effect can eradicate resisting and/or quiescent leukemic or tumor cells accounting for reduced rates of relapse in patients undergoing allogeneic HSCT.'(1) However, donor-derived T cells can also target normal cells of the recipient resulting in graft-versus-host disease (GvHD). (11,12)

1.2 Indications of HSCT

'Hematopoietic stem cell transplantation is used primarily for hematologic and lymphoid cancers but also for many other disorders.'(1) Almost 20.000 autologous transplantations were performed in Europe in 2012, two thirds for multiple myeloma or non Hodgkins's lymphoma (see figure 1 and 2). (14)

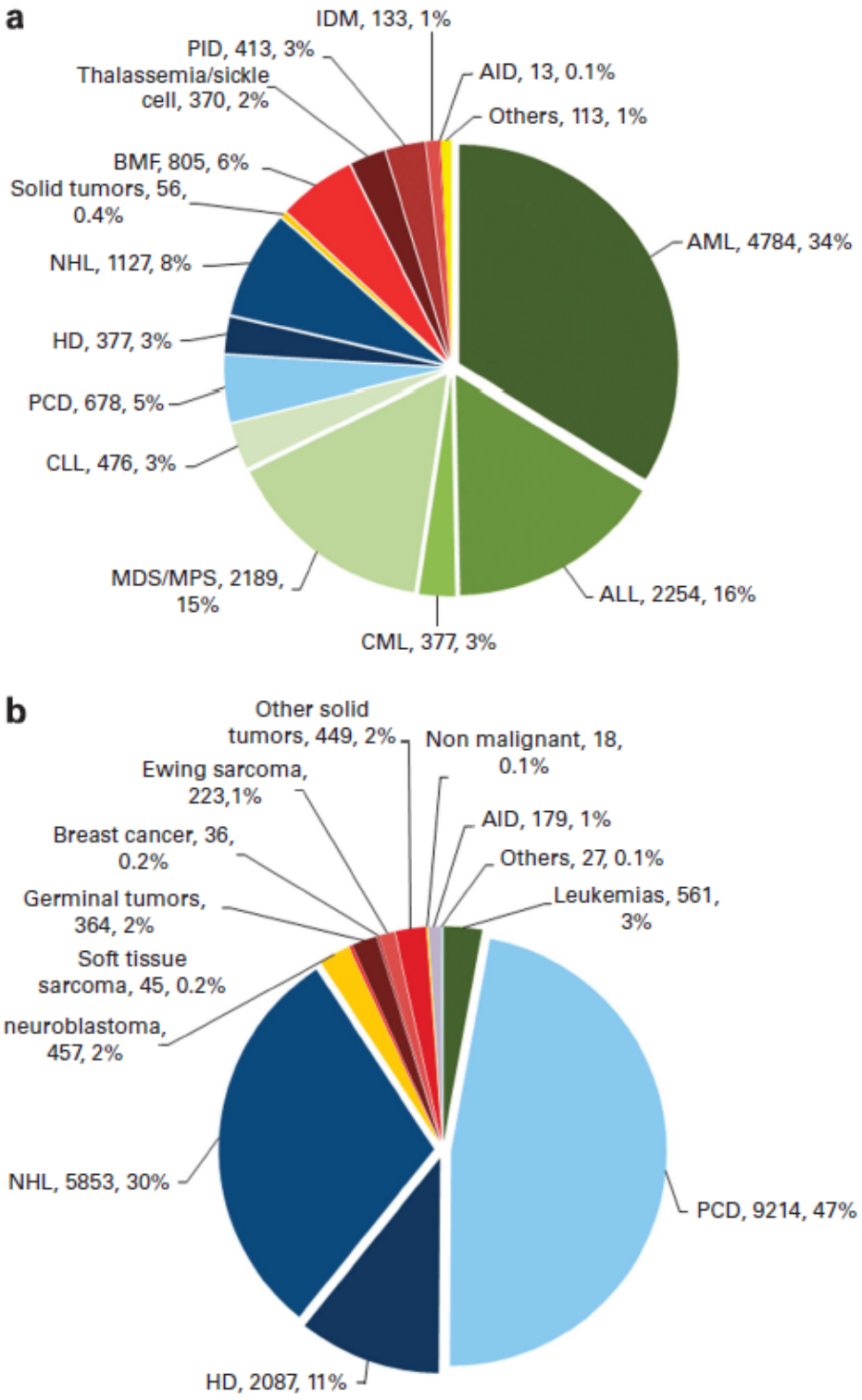

Figure 1. Absolut numbers and relative proportions of indications for an HSCT in Europe in 2012. (a) Proportions of disease indications for an allogeneic HSCT in Europe in 2012. (b) Proportions of disease indications for an autologous HSCT in Europe in 2012. (14) Abbreviations: AID= autoimmune deficiencies, ALL= acute lymphoid leukemia, AML= acute myelogenous leukemia, CLL= Chronic lymphoid leukemia, CML= chronic myelogenous leukemia HD= Hodgkin lymphoma, IDM= Inherited disorders of metabolism, MDS/MPS= myelodysplastic syndrome/myeloproliferative syndrome, PCD= plasma cell disorder, PID= primary immune deficiensis, NHL= non Hodgkin lymphoma

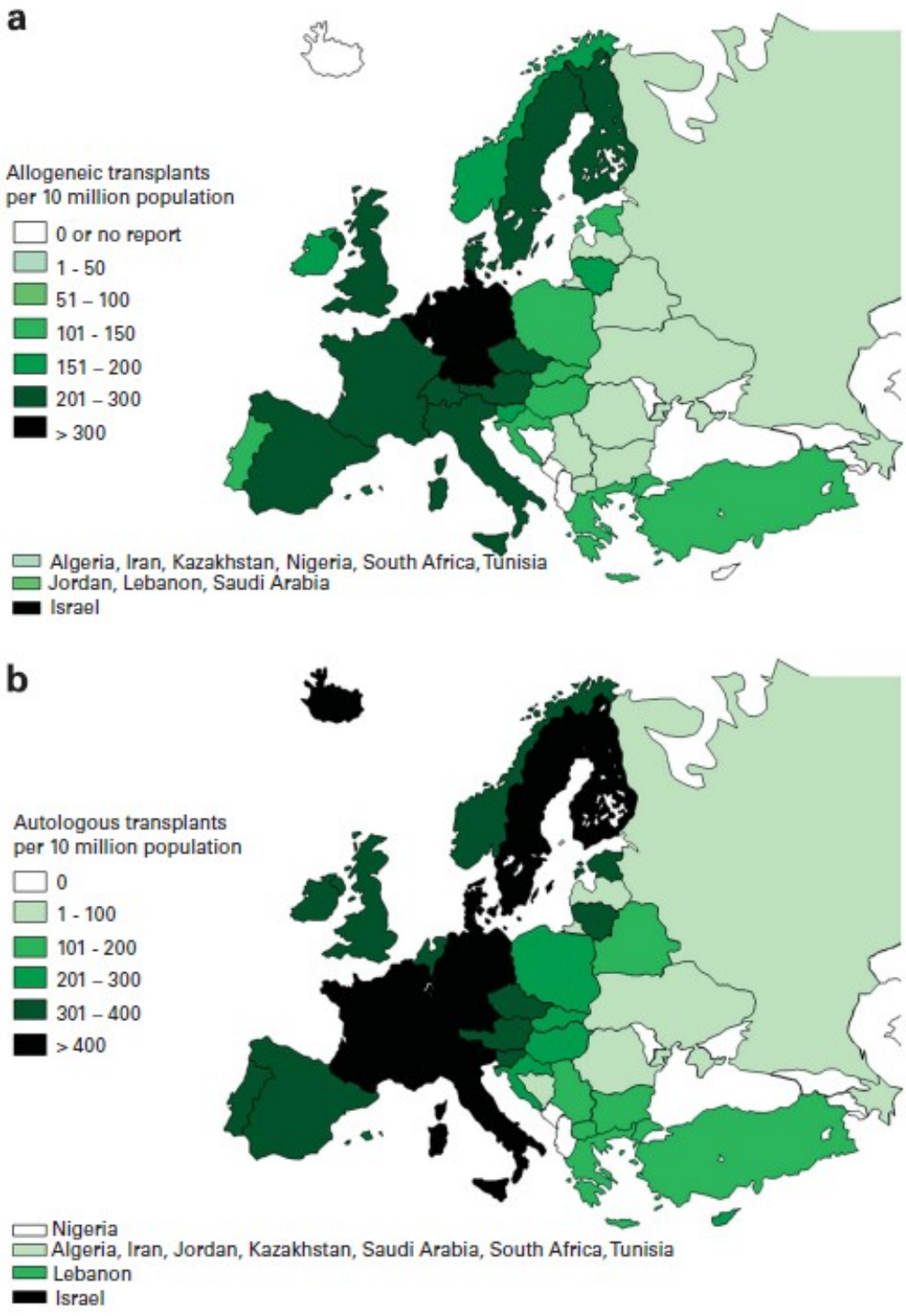

Figure 2. Transplant rates in Europe (=total number of HSCT per 10 million inhabitants) by participating country. (a) Allogeneic transplant rates/10 million population in 2012. (b) Autologous transplant rates/10 million population in 2012. (14)

The use of allogeneic transplantation has also been rising during the last three decades. ‘More than 14.000 allogeneic transplantations were performed in Europe in 2012, nearly half for acute leukemias.’(1) Regarding Austria 483 HSCT were performed in 2012, 300 autologous and 182 allogeneic transplantations including adults and kids. (13) Especially in patients with high risk and advanced haematological diseases allogeneic HSCT became the standard of care. The main indications for allogeneic HSCT are hematologic neoplasms derived from hematopoietic stem or progenitor cells, such as acute leukemias, but also benign congenital diseases of the bone marrow and the immune system. In these cases the patient´s hematopoiesis is completely replaced by HSC derived from a suitable allogeneic donor. The most common diseases are listed below. (5)

	disease	comment
Benign	aplastic anemia	Severe progression of the disease, ineffective immunosuppressive therapy
	Paroxysmal nocturnal hemoglobinuria	Severe progression
Malignant	acute myeloid leukemia	Depending on risk profile and state of remission
	acute lymphatic leukemia	Depending on risk profile and state of remission
	Myelodysplastic syndromes	In high risk patients, low risk patients with severe thrombocytopenia
	Chronic myelogenous leukemia	Ineffective therapy with tyrosin kinase inhibitors
	osteomyelofibrosis	In risk patients
	chronic lymphatic leukemia	In high risk patients
	non-Hodgkin lymphomas	In high risk patients
	multiple myeloma	In high risk patients

Table 1. Indications for allogeneic stem cell transplantation in hematologic diseases in adults (5)

1.3 Stem Cell Sources

'Traditionally hematopoietic stem cells were harvested from the posterior iliac crest.' (4) More recently there are two other sources of hematopoietic stem cells. It is possible to obtain the HSC from peripheral blood or cryopreserved umbilical cord blood.' (4)

1.3.1 Bone marrow

HSC physiologically grow in the bone marrow and about 0.1%of all bone marrow cells are HSC. Bone marrow is obtained via repetitive biopsies of the iliac crest. About 1000ml of bone marrow blood are needed, which should result in a total yield of 2-3x10^8 nucleated bone marrow cells per kg bodyweight of the recipient. General anaesthesia of the donor is needed, but serious side effects are rare. However, due to the need of intubation and general anaesthesia bone marrow as the source of HSC is not widely used any more. (1,11)

1.3.2 Peripheral blood

Although HSC normally reside in their specific niches in the bone marrow, they sometimes leave their niche and patrol in the peripheral blood. Therefore, about 0.05% of all leukocytes in the peripheral blood are CD34+ cells containing HSC. With the use of recombinant granulocyte colony-stimulating factor mobilization of HSC to the peripheral blood can be stimulated, which results in sufficient numbers of HSC to be obtained by leukapheresis through the antecubital veins. As an alternative drug for HSC mobilization the CXCR4 antagonist plerixafor is available. In total, 2-8x10^6 CD34+ cells per kg bodyweight of the donor are needed to give full hematopoietic reconstitution after transplantation. Besides the more comfortable way of HSC collection another advantage of peripheral blood as source of HSC is a more rapid hematopoietic reconstitution. 'However, peripheral blood HSC grafts, which contain more T cells than bone marrow does, increase the incidence and severity of chronic GvHD.' (1)

1.3.3 Umbilical cord blood

In contrast to peripheral blood of adults, umbilical cord blood (UCB) contains a relatively high number of HSC: about 0.1-2% of all leukocytes are CD34+ cells. Therefore, UCB represents an attractive source of HSC. Immediately after birth, UCB and rest blood of the placenta is conserved, frozen and stored in so called cord blood banks. (11) 'If the transplantation is urgent or if a suitable donor is not found, cord blood, which can be procured both easily and safely, can be used'. (1) Details on UCB are given in chapter 1.5.5.

1.4 Graft types

1.4.1 Autologous HSCT

In the case of autologous stem cell transplantation the patient´s HSC are isolated prior to the conditioning procedure and stored in liquid nitrogen. After myeloablation with chemotherapy and/or irradiation the collected HSC are thawed and reinfused to rescue hematopoiesis. Transplantation of autologous HSC is routinely used in patients with multiple myeloma and relapsed lymphoma. (12,15)

1.4.2 Allogeneic HSCT

HSC are obtained from a relative (most commonly an HLA matched sibling) or an unrelated person, who has been identified from a world-wide registry of people willing to donate bone marrow of peripheral blood HSC. This type of stem cell transplantation has become feasible through the discovery of human leucocyte antigen (HLA) typing. The HLA system or major histocompatibility complex (MHC) is a group of molecules containing more than 200 genes, which are central to the function of the immune system. ‘The primary role of HLA molecules is to present peptide to T-cells enabling them to recognise and eliminate foreign particles present in an individual.’ (4) ‘The HLA system displays extensive polymorphisms most likely due to the need for the immune system to keep up with and control infectious pathogens.’ (4) Virtually no human being has the same composition of MHC molecules (besides monozygotic twins). Despite this massive diversity it is possible to compare MHC molecules and identify compatible donors. Currently, in most patients matching is performed for both alleles on six HLA loci (HLA-A, -B, -C, -DR, -DQ, -DP). The higher the diversity is between donors and recipients major histocompatibility antigens, the higher is the risk for development of GvHD. Since about 70% of patients lacking a related donor and can’t find a suitable unrelated donor, alternative graft sources, such as umbilical cord blood (UCB) and haplo-identical donors, have become more and more attractive. (6,7,12,15)

1.4.3 Syngeneic HSCT

In this rare case HSC can be obtained from a genetically identical (monozygotic) twin. The advantage is that the donor´s HSC are genetically identical with the recipient. This results in a low risk of GvHD, but increased risk of relapse.

1.5 Allogeneic graft types

1.5.1 Matched related donor (MRD)

Typically the donor is a brother or a sister with fully matching HLA antigens, implying a compatible tissue type. It is known as sibling transplant or sibling allograft. (15)

1.5.2 Matched unrelated donor (MUD)

The donor is unrelated to the patient, but has a full match in all HLA loci analysed resulting in a compatible tissue type (15)

1.5.3 Mismatched unrelated donor (MMUD)

A mismatched unrelated donor displays one or two variations of the HLA loci compared to the recipient leading to a markedly increased risk of GvHD. (6,15)

1.5.4 Haplo-identical donor

In this case an only partially HLA-compatible family donor (parents, sibling or child) is selected. The immediate availability is a big advantage but this method is limited due to a high risk of graftment failure, GvHD, relapse and slow immune recovery.

1.5.5 Umbilical cord blood (UCB)

Umbilical cord blood can be used in patients who cannot find a matched unrelated donor. This is true for people with rare HLA constellations or patients from minority populations, which are underrepresented in the world-wide donor registry. The huge advantage of cord blood is the low rate of immunoreactive T-cells, compared to the richness of CD34+ cells. The common hypothesis about the low rate of immunoreactive T-cells is that cord blood obtained stem cells are more immunologically naive as cells from adult bone marrow or peripheral blood. (3)

Hence HLA incompatibility is tolerated to some extent and the risk of GvHD is limited despite mismatched HLA loci. Furthermore, UCB is stored in UCB banks and therefore immediately available. There is no risk for the mother or the child while obtaining the cells. To reduce the risk of transmitting infections (e.g CMV) the cord blood is tested and HLA typed in the frozen state. Due to the low numbers of CD34+ HSC in total, the main disadvantage is delayed engraftment which sometimes can even result in engraftment failure. This problem leads to high risk of infections with increased transplantation related mortality/morbidity (TRM). To overcome this problem of low HSC numbers in adults, two UCB units are often used to reach accurate HSC dose. (6)

1.6 Conditioning regimens

While in the autologous setting the conditioning regimens only aim for eradicating all malignant cells before HSC rescue, chemotherapy with or without irradiation is needed to suppress the patient's bone marrow and immune system before allogeneic HSCT. Thus, conditioning treatment allows engraftment of the donor's HSC and prevents graft failure. It also aims for eradication of the malignant cells, but is also intended to trigger an antileukemic or antitumor immune response. Malignant tumor cells undergo cell death during chemotherapy and irradiation, 'which results into a flood of tumor antigens.' (1) In turn leading to an immune response resulting in proliferation of leukemia-specific T-cells. Optimally, these T-cells can then eradicate the remaining malignant cells.

In many cases the conditioning regimen includes total body irradiation, which is myelo-and immunosuppressive. In addition, it may reach sites that are not affected by chemotherapy.' (1) Thus, the standard preparation since the 1980s has been fractionated total body irradiation combined with the chemotherapeutic agent cyclophosphamide. High dose chemotherapy (cyclophosphamide combined with busulfan) without radiation also 'proved effective in treating acute myeloid leukemia'.(1) Due to substantial toxicity such myeloablative regimens can mainly be used in patients under 55years without co-morbidities. However, within the last 15 years non-myeloablative or reduced-intensity conditioning (RIC) regimens have been developed allowing the treatment of older patients to the age of even 75

years. Primarily immunosuppressive, in these cases it depends on the graft to eradicate cancer. (1) It has been shown that the engraftment is reached without high toxicity, and neutropenia as well as thrombocytopenia are mild.

Common chemotherapeutic substances used with non-myeloablative or RIC regimens are busulfan, cyclophosphamid, fludarabine with or without combination of TBI. Common immunosuppressants applied with allogeneic transplantation are anti-thymocyte globulin (ATG), cyclosporin A (CsA), methotrexate (MTX) and mycophenolatemofetil (MMF). (6, 12)

1.7 Infections as frequent complications after HSCT

Besides GvHD, infections are the main cause of complications after HSCT resulting in significant morbidity and treatment-related mortality (TRM).

There are three stages of hematopoietic and immune recovery in the post transplant period: the early recovery or pre-engraftment phase encompassing the first two to four weeks after transplantation, the mid and late recovery phase. Due to stage-specific disturbances in immune functions, each phase has its particular microorganisms causing severe infections. (16)

1.7.1 Pre-engraftment phase

In the first 14-30 days after HSCT patients suffer from pancytopenia (neutropenia, anemia and thrombocytopenia) and mucosal damage. Due to neutropenia or rather agranulocytosis susceptibility to bacterial infections is very high. There are various origins for possible infections like the central venous line (CVL), Port-a-caths, and the, prior due to conditioning regimens injured, gastrointestinal mucosa. Preventive methods like hand hygiene, isolation in a single room, daily washing with aseptic agents, low germ or sterile food, prophylactic antibiotics, antiviral and antifungal drugs are needed. Although needed, antibiotics as prophylactic agents result in the emergence of resistant bacteria. (8,19) In the pre- engraftment phase patients suffer from neutropenia and mucosal damage.

Mucositis is the most common complication of myeloablative preparative regimens and methotrexate.' (1) 90% of infections are bacterial, overall Gram-positive bacteria (a chart of the most common Bacteria is listed below). Candida and aspergillus can also occur. (8,16)

Gram-positive	Gram-negative
Coagulase- negative Staphylococcus	Escherichia coli
Staphylococcus aureus (incl. MRSA)	Klebsiella
Enterococcus (incl VRE)	Enterobacter
Streptococcusviridans	Pseudomonas aeruginosa
Pneumococcus	Citrobacter
Streptococcus pyogenes	Acinebacterspp
Corynebacterium	Stenotrophomonas maltophilia
Clostridiumdifficile	

Table 2. Common bacterial pathogens in FN (8)

1.7.2 Mid recovery

In the mid recovery phase cellular and humoral immunodeficiency are predominant. Bacterial infections are decreasing whereas viral (especially cytomegalovirus (CMV), herpes virus) and fungal (aspergillus, candida) infections are seen. (16)

1.7.3 Late recovery

In the late recovery phase aspergillus infections predominate. Chronic GvHD leads to a low number of T helper cells (CD4) and infections with encapsulated bacteria can also occur. (16)

1.7.4 Febrile neutropenia

Fever and febrile neutropenia is another feared complication in patients undergoing HSCT. Due to aggressive radio and chemotherapies the patient's immune system is altered and becomes vulnerable. At least one half of neutropenic patients who become febrile have an established or occult infection and at least one fifth of patients with neutrophil counts of <100cells/mm³ have bacteremia. (17) Hence febrile neutropenia is afflicted with a high mortality risk about 11% and in leukemic patients even 18%.(8) The general definition of the

European society for Medical Oncology is: temperature 1x>38,5°C or 2x38°C over 2h and ANC<0,5x10^9/l or ANC presumably falling below.
The etiology of febrile neutropenia has been changing over the last decades, which makes an appropriate antibiotic therapy more difficult. While in the 1950s Staphylococcus aureus was the most common pathogen causing infections, in the following years Gram-negative bacteria e.g Pseudomonas aeruginosa became more frequent. During 1980 and 1990 Gram-positive bacteria, most commonly coagulase negative staphylococci, enterococci and S. aureus, recurred. Presently, Gram-negative bacteria are emerging again and with respect to Gram- positive microorganisms, coagulase negative staphylococci and enterococcus species are increasingly indentified. Furthermore 'new' microorganisms and resistant Gram-positive pathogens are evolving. However in febrile neutropenia the patients are prone to several infections and adequate antibiotic therapy is always indicated. (18)

Depending on the absolute neutrophil count patients can be stratified into low and high risk categories. In low risk patients (neutropenia 100-500/µl; duration of neutropenia<10days) a combination of oral broad-spectrum-antibiotics (amoxicillin/clavulanic acid and ciprofloxacines) is used.
In high-risk patients (neutropenia<100µl; neutropenia>10days) an intravenously broad spectrum antibiotic (piperacilline/tazobactame or cephalosporines of the third and fourth generation) is applied. If this treatment is not successful, carbapenems are another option. In case of complications or resistances fluorchinolones, aminoglycosides or glycopeptides (teicoplanin, vancomycin) are used. For infections with Gram-positive, Methicillin-resistant Staphylococcus aureus (MRSA) vancomycin, linezolid, daptomycin or teicoplanin are an option. In case of vancomycin resistant enterococci (VRE) only linezolid and quinupristin/dalfopristin are approved by the food and drug administration (FDA). Since signs and symptoms of inflammation can be minimal or absent in severely neutropenic patients a search for subtle symptoms should be undertaken. A microbiological screening is of utter importance including blood cultures. (8,12, 17,20)

1.8 Graft versus host disease (GvHD)

Besides infections the graft versus host disease (GvHD) is the most common important complication after allogeneic HSCT. It can occur despite perfect HLA matching and immunosuppressive therapy, although the risk is dramatically increasing with HLA incompatibilities. Basically the GvHD emerges through the reaction of immuno-competent T-cells from the donor. These cells recognise not only ‘foreign’ malignant cells but also healthy cells of the recipient. Through the contact with the antigen the T-cells are activated, start proliferating and an immunological response is triggered eventually resulting in the attack of normal tissue of the recipient. Acute GvHD is defined to occur within 100 days after transplantation, while chronic GvHD develops after day 100. The concerned organs in acute GvHD are the skin (e.g erythema), gastrointestinal tract (severe diarrhoea), and liver (increasing liver enzymes, icterus). In chronic GvHD skin (scleroderma, fibrosis), mucous membranes (siccasymptomes), liver and lung are affected. (1,11,12)

1.9 Linezolid

Linezolid (trade name Zyvox or Zyvoxid) is a relatively new antibiotic drug belonging to the group of oxazolidinones. Approved in the USA in April 2000 it has become an important agent for the treatment of infections with aerobic and anaerobic Gram-positive organisms including otherwise resistant bacteria, such as vancomycin resistant enterococci, methicillin resistant Staphylococcus aureus (MRSA) and penicillin resistant pneumococci. Linezolid is a bacteriostatic agent; Its complex mechanism is to inhibit the translation phase of bacterial protein synthesis through binding to the 50S ribosomal subunit within the 23s rRNA peptidyltransferase. (21,22,23)

1.9.1 Indication

It is the only oral oxazolidinone approved for the treatment of MRSA and VRE infections. In addition it is indicated in nosocomial pneumonia, community-acquired pneumonia, uncomplicated and complicated skin and skin structure infections. (24)

1.9.2 Pharmacokinetics

600mg linezolid can be administered intravenously or – due to its good bioavailability - orally via film-coated tablets and in oral suspension twice daily. Linezolid has a plasma elimination half live of 3.4-7.4h and is metabolised in the kidney but also in a non-renal mechanism into two active metabolites, aminoethoxyacetic acid (metabolite A) and hydroxyethyl glycine (metabolite B). The metabolites however have not shown significant anti-microbial activity or toxicity. In case of mild to moderate renal or hepatic impairment no dose adjustments are needed. Patients with severe renal insufficiency should be treated with care due to the higher exposure to the two active metabolites. There are no diversities in pharmacokinetics between older and younger healthy volunteers and no between men and women. High fat meals delay and decrease the Cmax, but there is no impact on the absorption and distribution. Also enteral feeding has no affect. Critically ill patients with severe sepsis in need of an intensive care unit do not need a dose adjustment. (23,24,25)

1.9.3 Side effects of linezolid

The most common side effects (reported in >2% of patients) are diarrhea, nausea and headache. (26)

Since linezolid is a reversible inhibitor of monoamino-oxidase (MAO) A and B, the combination of Zyvox with selective serotonin-reuptake inhibitors or MAO inhibitors can lead to the serotonin syndrome. (27) In combination with adrenergic agents e.g. tyramine linezolid shows a potential for a reversible pressor response. Furthermore linezolid has been associated with hematologic toxicity in the general population. The reported hematologic side effects include anaemia, thrombocytopenia, leukopenia, and pancytopenia. Particularly patients receiving linezolid for more than two weeks and receiving bone marrow suppressing agents are at risk. In addition, there are concerns in patients with pre-existing myelosuppression. A monitoring of the complete blood count should be done every week.(24,28) In a retrospective case-controlled study from Cohen et al. conducted from 2000 until 2007 linezolid and vancomycin were compared due to differences in the times to neutrophil and platelet engraftment in patients receiving allogeneic HSCT. No negative impact of linezolid on hematologic engraftment was found. (28) However, the authors claimed that larger studies are needed. In a randomized, double blind study by Jaksic et al. linezolid and vancomycin were compared in febrile neutropenic patients with cancer. There were no differences between the groups in platelet counts but a transient delay in absolute neutrophil counts not affecting the mean duration of treatment. (29) In a third study Smith et al. compared linezolid to other antibiotic agents in patients with cancer and neutropenia. They failed to show differences in terms of hematologic side effects between patients with neutropenia and the overall compassionate use population. (9) Thus, none of these three studies could show any increased risk of hematologic adverse effects in patients with neutropenia or patients undergoing bone marrow transplantation. (30)

1.9.4 Bacterial resistance to linezolid

Resistant bacteria to linezolid are relatively uncommon in comparison to other antibiotics. Nevertheless infections with linezolid resistant enterococci have been reported. Therefore, due to the importance of linezolid in treatment of VRE infections it is crucial to closely monitor emerging resistances. (31) While the first resistance was discovered in E. faecium infections, soon resistances in staphylococci and other enterococci were reported. For example, in a case report in 2004 from Dibo et al. (25) a linezolid resistant Enterococcus faecalis in a cord blood transplant recipient was identified. Different mutations in the genes encoding 23s rRNA were associated with resistant bacteria. (24)

1.10 Purpose of the study

As outlined above there are only very few studies published, investigating the impact of linezolid use on hematologic recovery in HSCT. In particular, there are no data available about risks on hematologic recovery after umbilical cord blood transplantation. The low number of transplanted HSC with UCB may render such patients very sensitive to myelosuppressive side effects of antibiotics. On the other hand, due to prolonged neutropenia and increasing infections with resistant bacteria, such as MRSA and VRE, an appropriate antibiotic therapy is inevitable. Linezolid is among the few antibiotic agents highly active in persistent febrile neutropenia as well as infections caused by MRSA or VRE. Thus, the knowledge about potential side effects especially myelosuppression in this patient population is of utmost importance.

The aim of this study is to analyse all relevant clinical, biochemical and microbiological data of patients having undergone umbilical stem cell transplantation at the Division of Hematology at the Medical University of Graz in order to systematically elucidate potential myelosuppressive side effects as well as emerging resistance to linezolid.

1.11 Objectives in short

- To determine the impact of linezolid treatment on hematologic engraftment in patients undergoing umbilical cord blood- SCT
- To determine the occurrence of linezolid resistant bacteria in patients treated with linezolid after umbilical cord blood SCT.

2 Material and Methods

2.1 Patients

This retrospective study included all patients having undergone umbilical cord blood stem cell transplantation between December 2001 and March 2013 at the Division of Hematology, Medical University of Graz.

2.2 Study design

A retrospective analysis of all relevant patients' files was performed. End points were defined as hematologic engraftment of neutrophils and platelets as well as determination of linezolid resistant bacteria.

The following personal and medical data from these patients were retrieved from the medical records and the electronic documentation program MEDOCS. Age at date of primary diagnosis, age at transplantation, sex, disease, date of relapse, bodyweight at transplantation, immunosuppressive therapy, conditioning regimen, dose of CD34+ cells infused per kg bodyweight, number of received grafts, administration of G-CSF (yes/no), days on linezolid therapy, number of days to platelet and neutrophil recovery (see also below), hepatic toxicity defined by total bilirubin levels >3mg/dl, renal insufficiency defined by creatinine levels> 2mg/dl, number of days with fever > 38°C, overall survival and disease-free survival.

In addition, microbiological data were determined. These data included the number of days with fever > 38°C as well as the numbers of (positive) blood cultures, number of swabs of suspected infection sites, number of stool cultures, number of other microbiological cultures as well as the spectrum of identified bacteria including their sensitivity to linezolid.

After anonymization the collected data were entered into a data collection sheet using Microsoft EXCEL.

2.2.1 Definitions

Time to neutrophil engraftment (ANC500 and ANC1000)

ANC500 and ANC1000 were defined as the number of days from the date of stem cell transplantation to the first date of a stable absolute neutrophil count >500/mm^3 and >1000/mm^3.

Time to platelet engraftment (PLT20, PLT50, PLT100)

PLT20, PLT50 and PLT100 were defined as the number of days from the date of stem cell transplantation to the first day of a stable platelet count PLT>20000/mm^3 PLT >50000/mm^3 and PLT>100000/mm^3, respectively.

2.3 Statistical Analysis

R 3.1.1 (www.r-project.org) and Microsoft Excel were used for statistical analysis. Statistical significance was defined as a p- value < 0.05. Overall survival was estimated using the Kaplan-Meier method. Competing risk analysis was used to estimate the probability of platelet and neutrophil engraftment. Competing risk analysis is necessary in order to account for the fact that some patients died before recovery.

Confidence limits for survival and recovery estimates were calculated symmetrically at the log minus log scale. The same method was applied for the median survival times and median times to recovery.

To identify risk factors for delayed and incomplete neutrophil and platelet recovery, several parameters including linezolid treatment were assessed by univariate and multivariate analyses using the Cox regression model. Multiple recovery events were analysed simultaneously by clustered survival analysis, treating patients as clusters. The comparison of two groups with respect to survival is a special case of score test within the Cox model, which is otherwise known as log-rank test. Furthermore, the incidence of infections by linezolid-resistant bacteria was calculated.

The study was approved by the Ethics committee of the medical University Graz and was conducted in accordance with the Code of Ethics of the World Health Organization. The study character was non interventional hence no prior patient consent was needed.

3 Results

3.1 Patients characteristics

35 patients (12 females (32.4%) and 23 males (67.6%)) underwent umbilical cord blood stem cell transplantation in the time period between December 2001 and March 2013 and were therefore included in this study. Two patients were transplanted twice with UCB. One patient received a second transplantation due to graft failure, the other one was re-transplanted due to leukemia relapse. Since in both cases patients died within few days after the second transplantation, only the first transplantation was included in our analysis. Four patients received two umbilical cord blood units for transplantation. During the period of neutropenia after transplantation 28 patients (80%) were treated with linezolid while 7 patients (20%) did not receive this drug.

The median age at diagnosis for all patients was 42 years (range 19-65 years). In the linezolid group the median age was with 38 years (19-65 years), which was slightly but not significantly lower as in the no-linezolid group (51 years, 20-61 years).

In all patients the underlying diseases were hematologic malignancies with most of the patients suffering from acute myeloid leukemia (AML, n=18, 51.4%), seven patients had acute lymphoblastic leukemia (ALL, 20%),10 patients (28.6%) suffered from other hematologic malignancies including Hodgkin´s lymphoma, follicular lymphoma, diffuse large B cell lymphoma, myelodysplastic syndrome and multiple myeloma.
11 Patients had relapsed disease, 2 patients had even their second relapse prior to UCB-HSCT.

The median age at SCT was 42 years (19 –67 years). In the linezolid group the median age was 39 years (19 -67 years), which is again slightly lower than in the no-linezolid group (52 years, 20 -61 years).

The median interval between the date of diagnosis and the date of SCT was 14 months (range 4- 113 months). In the linezolid group the median was 14 months

(range 5- 113 months) and therefore the same as in the no-linezolid group (14 months, range 4- 29 months).
The median follow up time (date of transplantation to last contact or date of death) of all patients was 7 months (range 0- 144 months), the median follow up of surviving patients was 34.5 months (range 1- 144 months)

The patients´ characteristics between both groups were analysed by using the T-Test, however, no statistical difference was found.

Patients' characteristics are shown in the table below

	collective	Linezolid	no Linezolid	p value
number of transplantations	**35 100%**	**28 80%**	**7 20%**	
age date of diagnosis				
median	42	38	51	0,39
average	40,19	39,27	44,14	
range	19 - 65	19 - 65	20 - 61	
age date of transplantation				
median	42	39	52	0,5
average	41,70	40,97	44,86	
range	19 - 67	19 - 67	21 - 62	
months between diagnosis and transplantation				
median	14	14	14	0,39
average	21	22	14	
range	4 - 113	5 - 113	4 - 29	
Follow-UP (months)				
median	7	8	5	0,53
average	20,65	22,23	13,86	
range	0 - 144	0 - 144	0 - 40	
Follow-UP (months) surviving patients (n=14)				
median	34,5	36	33	0,37
average	44,00	49,27	24,67	
range	1 - 144	1 - 144	1 - 40	

Table 3. Patients' characteristics

3.2 Treatment characteristics

The median dose of CD 34+ cells infused per kg bodyweight in the linezolid group was 1.26 x 10^5 (range, 2.00 x 10^4 – 7.00 x 10^5) and in the no-linezolid group 1.90 x 10^5 (range, 6.00 x10^4 – 7.00 x 10^5)

Linezolid was given to a total of 28 patients due to persisting febrile neutropenia or infection with linezolid-sensitive bacteria. It was administered with a dose of 600mg twice daily according to the instructions. The median number of days on linezolid therapy after UCB transplantation was 16 days (range 3- 57 days).

In the linezolid group, G-CSF was administered in 15 out of 28 patients (40.5%), while in the no-linezolid group 3 out of 7 patients received G-CSF (42.9%).

Hepatic toxicity, defined as bilirubin>3mg/dl was found in 15 patients of the linezolid group (53.6%) and in 3 patients of the alternative group (42.9%). Renal insufficiency defined as creatinine>2mg/dl was seen in 7 patients of the linezolid group (25%) and in 3 patients of the alternative group (42.9%).

The treatment characteristics of both groups were compared using the T- Test and the Fishers exact test; again, no statistical difference was found.

	collective		Linezolid		no Linezolid		p value
n=35	**35**	**100%**	**28**	**80,00%**	**7**	**20,00%**	
CD34+cells							
median	1,44 x 10^5		1,26 x 10^5		1,90 x 10^5		0,67
average	2,33 x 10^5		2,26 x 10^5		2,62 x 10^5		
range	2,00 x 10^4 - 7,00 x 10^5		2,00 x 10^4 - 7,00 x 10^5		6,00 x 10^4 - 7,00x10^5		
days Linezolid							
median	1		1				
average	1		1				
range	3 - 57		3- 57				
G-CSF							
yes	18	51,43%	15	53,57%	3	42,86%	1,00
no	17	48,57%	13	46,43%	4	57,14%	
Bilirubin>3mg/dl							
yes	18	51,43%	15	53,57%	3	42,86%	0,69
no	17	48,57%	13	46,43%	4	57,14%	
creatinine >2mg/dl							
yes	10	28,57%	7	25,00%	3	42,86%	0,35
no	25	71,43%	21	75,00%	4	57,14%	

Table 4. Treatment characteristics

This boxplot figure shows the distribution of our patients' main data.

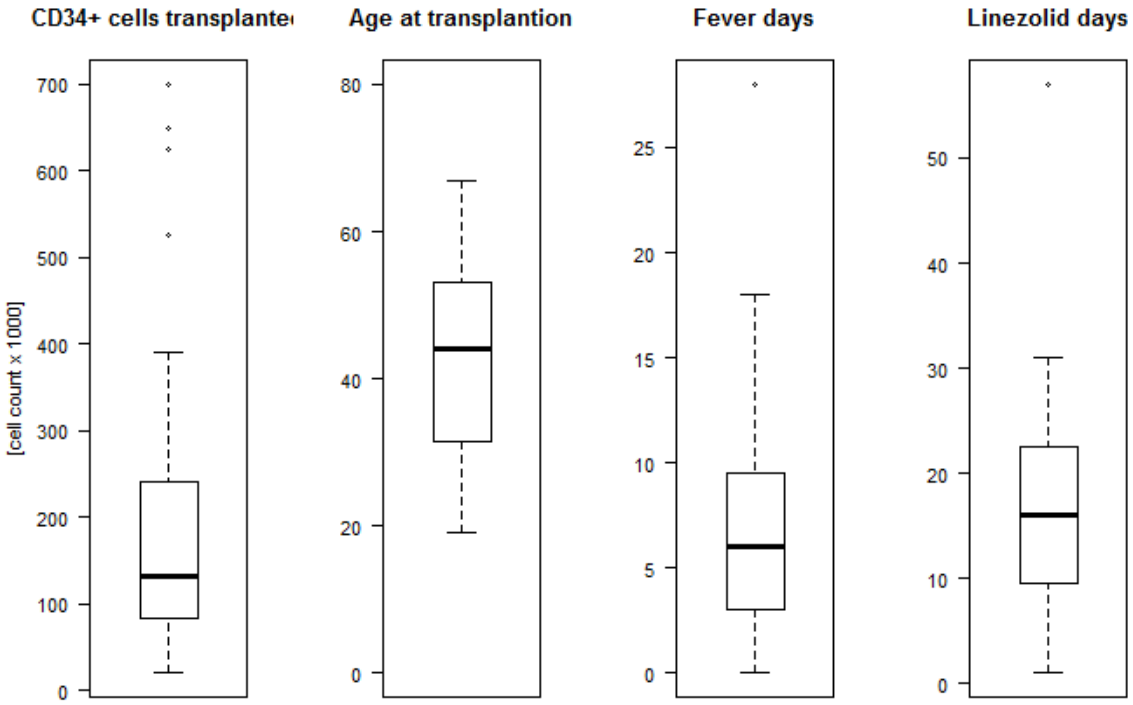

Figure 3. Distribution of patients' main data. Median of the CD34+ cells transplanted, age at transplantation, fever in days and linezolid in days.

Most patients received a conditioning regimen based on fractioned total body irradiation (fTBI) combined with chemotherapy. The most common conditioning regimen was the combination of fTBI, fludarabine and cyclophosphamide in 15 patients (42.9%). 6 patients (17.1%) received fTBI and cyclophosphamide, and 4 (11.4%) fTBI and etoposid. In 10 patients (28.6%) the conditioning regimen only contained chemotherapy including cytarabine, melphalan, carmustin and/or busulfan.

All patients received immunosuppressive therapy based on cyclosporin A (CSA), methotrexate (MTX), anti-thymocyte globulin (ATG) and/or mycophenolate-mofetil (MMF). The most common combination was CSA, MTX, ATG in 29 patients (82.9%). 3 patients (8.6%) received only CSA and MTX, two patients (5.7%) received CSA and MMF and one patient (2.9%) was administered CSA and ATG.

3.3 Survival data and causes of death

With a median follow up of 7 months (all patients) and 34.5 months (surviving patients) 21 out of 35 patients (60%) died after transplantation and 14 patients (40%) are still alive. The estimated overall survival after one year in all patients was 45% (95% confidence interval: 0.30%-0.6%) after 2 years 38% (95% CI: 0.2%-0.6%) and 31% after 3 years (95% CI: 0.2%-0.50%) (see Figure 4a). There was no difference in overall survival in patients receiving linezolid in comparison to patients without linezolid treatment (p= 0.94) (Figure 4b). The respective overall survival estimates after one, two and three years were 44%, 40% as well as 31% for the linezolid group and 51%, 34% and 34% for patients in the no linezolid group.

Regarding the causes of death, 11 out of 21 patients died of infections (52.4%), 9 patients died of progressive disease (42.9%) and one patient died of acute cardiac failure (4.8%).

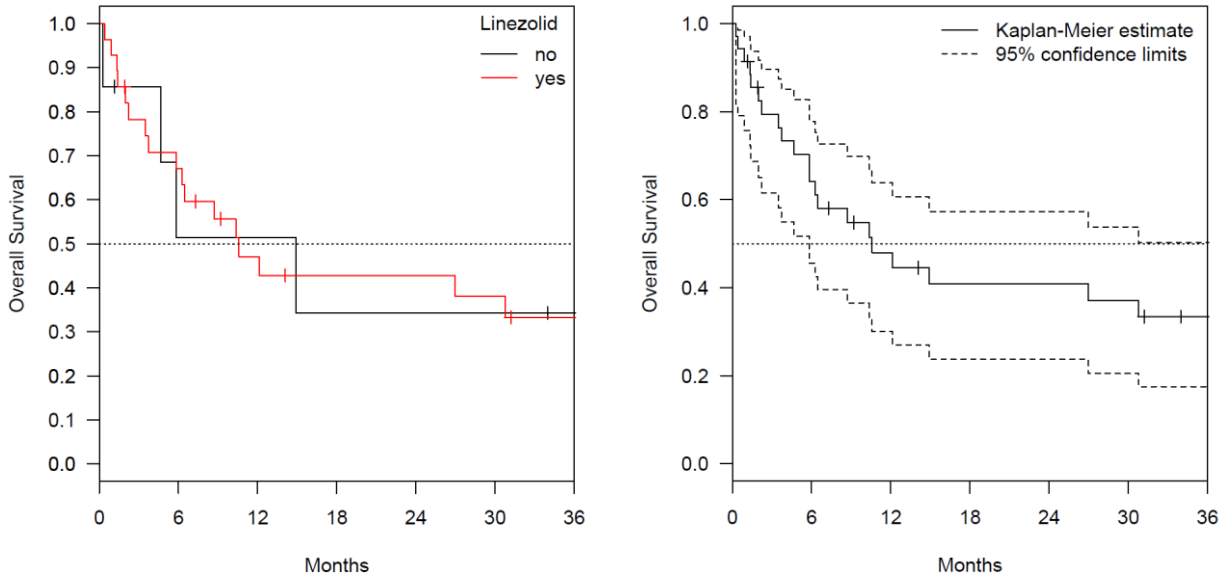

Figure 4. Kaplan-Meier estimate overall survival

3.4 Hematologic Engraftment

3.4.1 Neutrophil recovery

ANC500

30 out of all 35 patients (85.7%) were able to reach ANC500 with a median duration to ANC500 of 33 days (range, 18-67 days). 5 patients died before engraftment.

In competing risk analysis median time to grafting was 35 days (95% confidence interval CI: 28-40 days). The last patient without engraftment died after 67 days; at that time the estimated engraftment was 86% (CI: 68%-94%) and survival was 86% (CI: 75%-98%).That means that the engraftment curve and the survival curve did meet.

There was no difference regarding to linezolid treatment: While in the linezolid group 24 out of 28 patients (85.7%) were able to engraft to ANC500, in the no linezolid group 6 out of 7 patients (85.7%) achieved ANC500. The median duration to ANC500 in both groups was 32 days (range 18- 67 days) and 35 days (range 25- 52 days), respectively.

ANC1000

After a median duration of 42 days (range 22- 79 days) from transplantation 27 out of 35 patients (77.1%) achieved ANC1000. 8 patients died before engraftment.

In competing risk analysis median time to grafting was 45 days (95% CI: 41-67days).

The last patient without engraftment died after 30.8 months. At that time the estimated engraftment was 79% (CI: 59%-89%) and survival was 79% (CI: 66%-94%). That means that the engraftment curve and the survival curve again did meet. Two patients died without engraftment after the maximum time to ANC1000, one after 5.8 months and one after 30.8 months.

Again there was no difference between patients treated with or without linezolid (21 out of 28 patients (75%), median duration 42 days (range 22- 97 days) versus 6 out of 7 patients (85.7%), median duration 41 days (range 30- 63 days).

The statistical test for difference between no linezolid and linezolid with respect to both ANC recovery criteria was not significant (p = 0.41).
In the following plots (figures 6 for the whole cohort, and figures 8 broken down for the linezolid yes/no groups ANC (NEU)) estimated survival probabilities, i.e. death and grafting probabilities by this method are shown.

3.4.2 Platelet recovery

PLT20

28 out of 35 patients (80%) were able to reach PLT20 after a median duration of 35.5 days from transplantation (range 15- 61 days). In competing risk analysis median time to grafting was 41 days (CI: 34-54 days). The curves did not meet due to one patient having a long observation time without grafting (14.0 months); After the last event (death) 82% (CI: 63%-91%) of the patients had an engraftment and 85% (CI: 74%-98%) of the patients had survived.

In the linezolid group 23 patients displayed PLT20 engraftment (82.1%); median duration to PLT20 34 days, (range15- 61 days); in the no linezolid group 5 out of 7 engrafted (71.4%); median duration 36 days, (range 24- 47 days).

PLT50

After a median duration of 44.5 days (range 19- 121days) 25 out of 35 patients (71.4%) achieved PLT50.
In competing risk analysis median time to grafting was 55 days (CI: 44-83 days). After the last event (death) 77% (CI: 57%-88%) of the patients had an engraftment and 80% (CI: 67%-94%) of the patients had survived.

While in the linezolid group 20 out of 28 displayed PLT50 engraftment (71.4%;; median duration to engraftment 45 days (range 19- 105 days)), in the no linezolid group 5 out of 7 engrafted (71.4%; median duration to engraftment 43 days (range 31- 121 days)).

PLT100

In the whole cohort 21 patients (60%) were able to achieve stable PLT100 engraftment after a median of 55 days (range 26- 198 days).
In competing risk analysis median time to grafting was 102 days (CI: 61 days - not available). After the last event (death) 64% (CI: 43%-77%) of the patients had an engraftment and 70% (CI: 56%-87%) of the patients had survived.

In the linezolid group 16 patients (57.1%) engrafted (after a median of 54 days, range 26- 198 days), while in the no linezolid group 5 out of 7 patients engrafted (71.4%; median duration to PLT100 was 56 days, range 31-171 days).

In the following plots (figures 5 for the whole cohort, and figures 7 broken down for the linezolid yes/no groups) PLT estimated survival probabilities, i.e. death and grafting probabilities by this method are shown.
All data concerning hematologic engraftment of all patients are summarized in Table 5.

The statistical test for difference between no linezolid and linezolid with respect to all three PLT recovery criteria was not significant ($p = 0.33$).

In total, these data show that linezolid treatment did neither affect platelet nor neutrophil recovery after UCB-SCT in our cohort.

n=35	collective 35	100%	Linezolid 28	80,00%	no Linezolid 7	20,00%	p value
ANC500							
TX patients with engraftment	**30**	**85,71%**	**24**	**85,71%**	**6**	**85,71%**	0.79
median	33,00		32,00		35,00		
average	34,43		34,17		35,50		
range	18 - 67		18 - 67		25 - 52		
ANC1000							
TX patients with engraftment	**27**	**77,14%**	**21**	**75,00%**	**6**	**85,71%**	0.36
median	42		42		41		
average	43,74		43,95		43,00		
range	22 - 79		22 - 79		30 - 63		
PLT20							
TX patients with engraftment	**28**	**80,00%**	**23**	**82,14%**	**5**	**71,43%**	0.64
median	35,5		34		36		
average	38,25		38,43		37,40		
range	15 - 61		15 - 61		24 - 47		
PLT50							
TX patients with engraftment	**25**	**71,43%**	**20**	**71,43%**	**5**	**71,43%**	0.62
median	44,5		45		43		
average	52,19		50,67		58,60		
range	19 - 121		19 - 105		31 - 121		
PLT100							
TX patients with engraftment	**21**	**60,00%**	**16**	**57,14%**	**5**	**71,43%**	0.18
median	55		54		56		
average	74,24		73,63		76,20		
range	26 - 198		26 - 198		37 - 171		

Table 5 Engraftment. The p-values were calculated using the log-rank test; no statistically significant difference was found in engraftment.

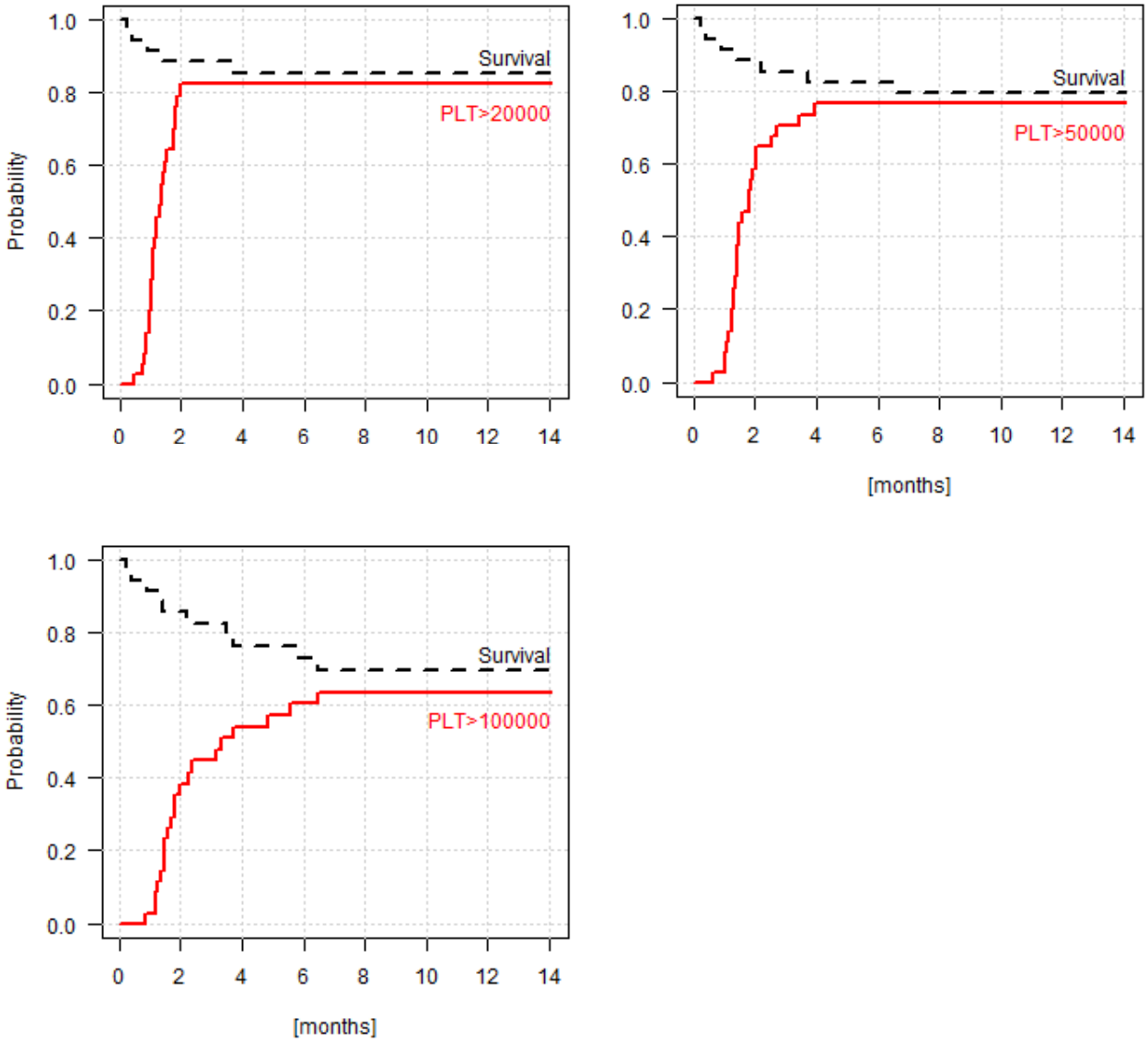

Figure 5 Competing risk model rebound PLT

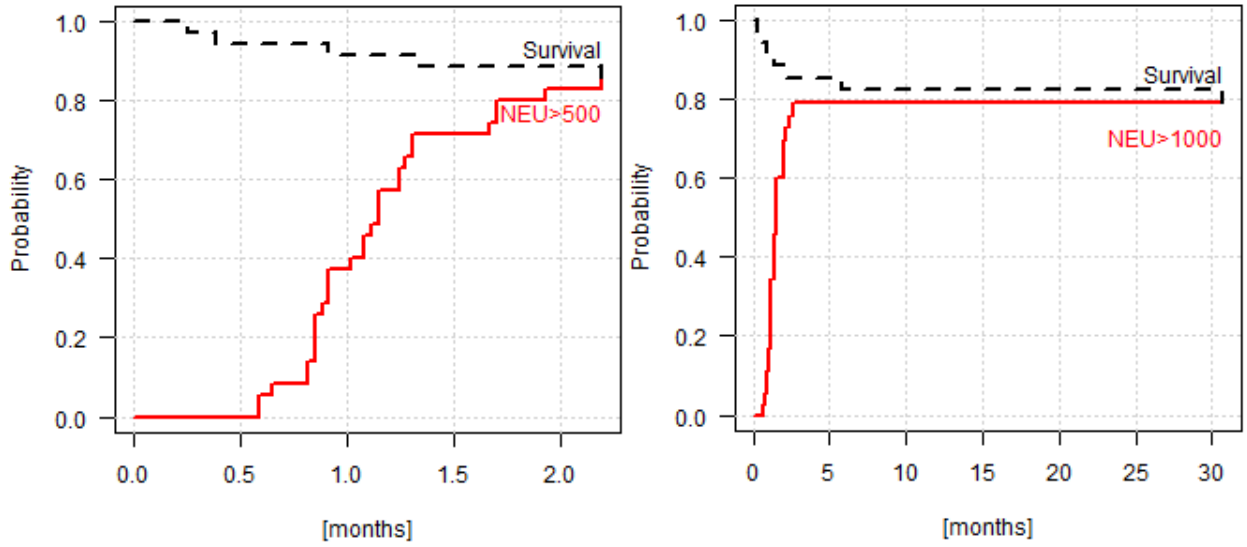

Figure 6 Competing risk model rebound ANC (NEU)

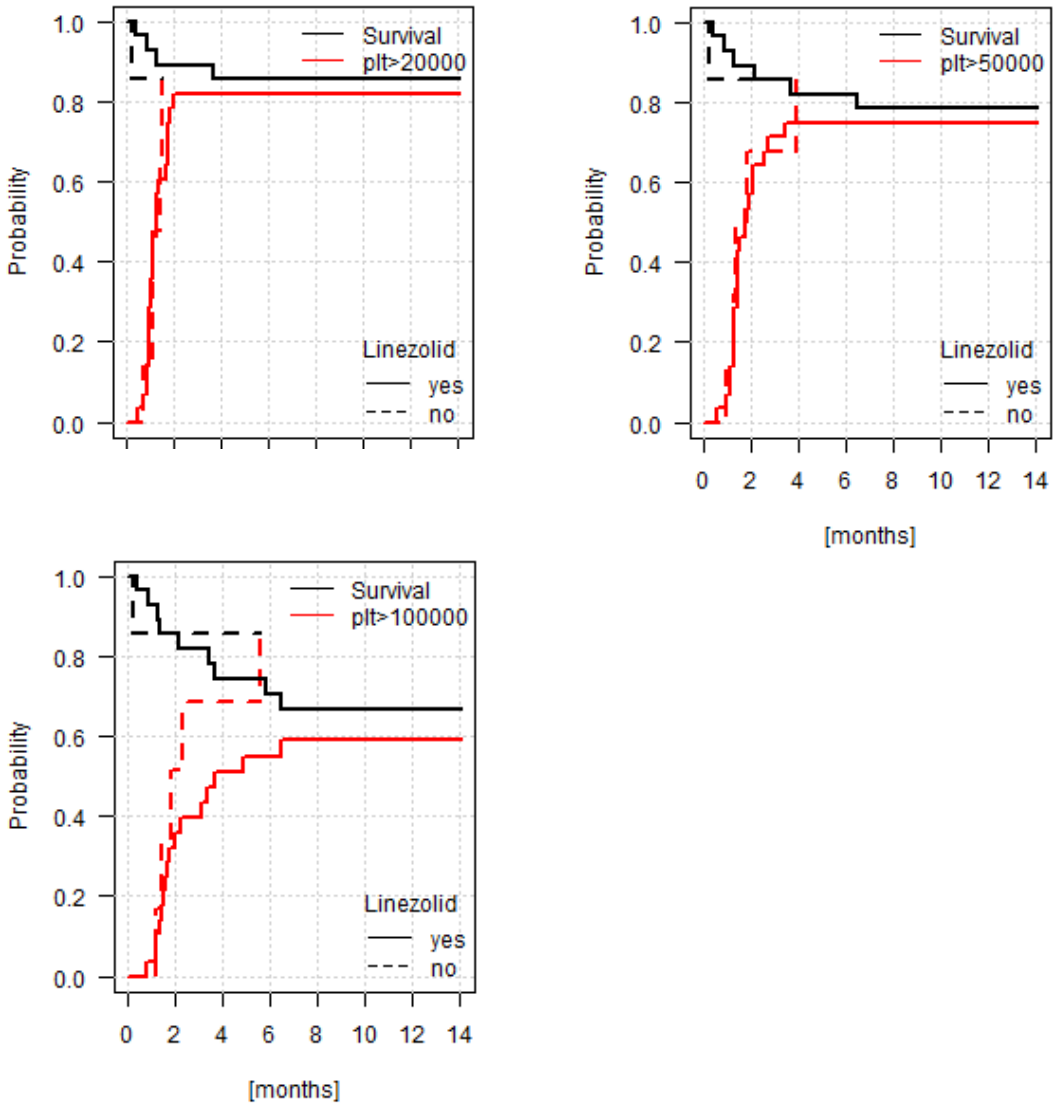

Figure 7 Competing risk model PLT linzolid yes/no

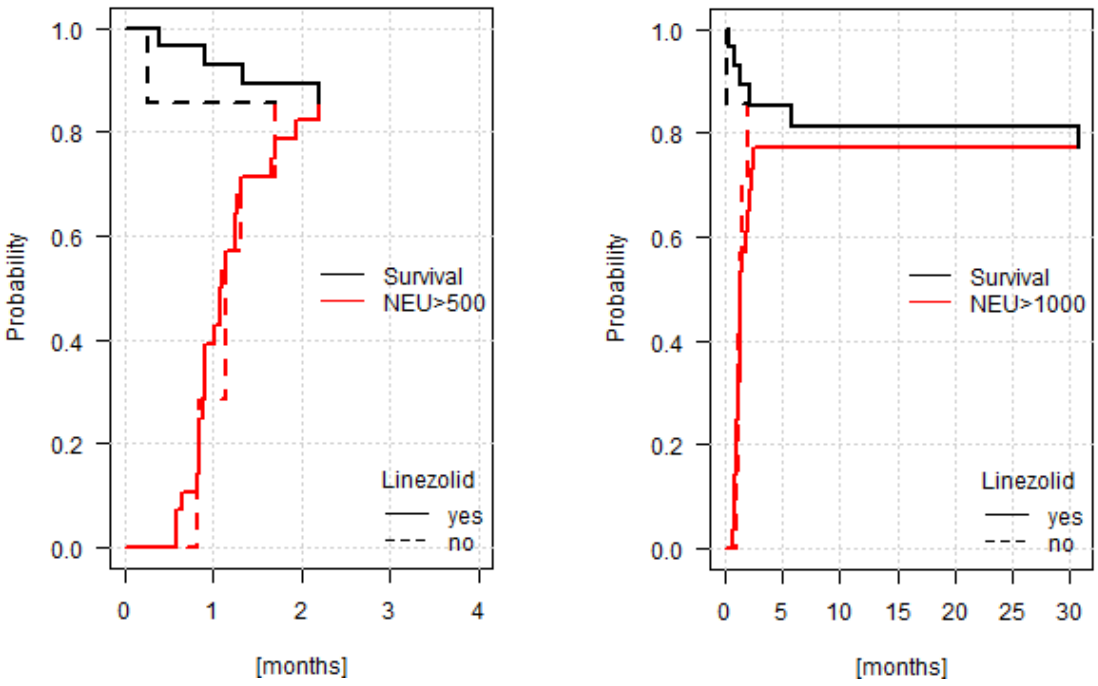

Figure 8 Competing risk model ANC (NEU) linezolid yes/no

3.5. Microbiological data

To determine the occurrence of linezolid-resistant bacteria we also analyzed all microbiological data available from the patients included in this study. These data encompassed information on blood cultures, routine surveillance cultures of nasal, throat, genital and rectal swabs, stool cultures and other cultures obtained during the hospital stay of the patients. Number of positive cultures and the nature of isolated bacteria as well as the corresponding antibiotic susceptibility test results were recorded (summarized data can also be found in Table 6).

Fever, which was defined as a temperature above 38° Celsius developed in 34 of 35 patients. The median duration of fever was 6 days (range, 0-28 days) in the linezolid group, and 3 days (range, 0- 12 days) in the no linezolid group.

In total 266 blood cultures were taken in the whole cohort, with 239 samples taken in the linezolid group (median of 8 cultures per patient, range, 1-16 days). Out of these 65 were positive (27.2%). The majority of isolated bacteria in this group were Gram-positive pathogens (51 isolates (21.3% of all blood cultures taken)) and all were linezolid sensitive. In the no linezolid group 13 out of 27 blood cultures taken were positive (48.2%). Again the majority of isolates were Gram-positive bacteria (n=9; 33.3%; see also table 6). Among these one isolated pathogen was a linezolid-resistant Enterococcus faecium. This enterococcus was already known before in the respective patient, since it had been identified in the stool cultures of this patient during induction chemotherapy for AML relapse before UCB transplantation while receiving linezolid treatment for persistent febrile neutropenia.

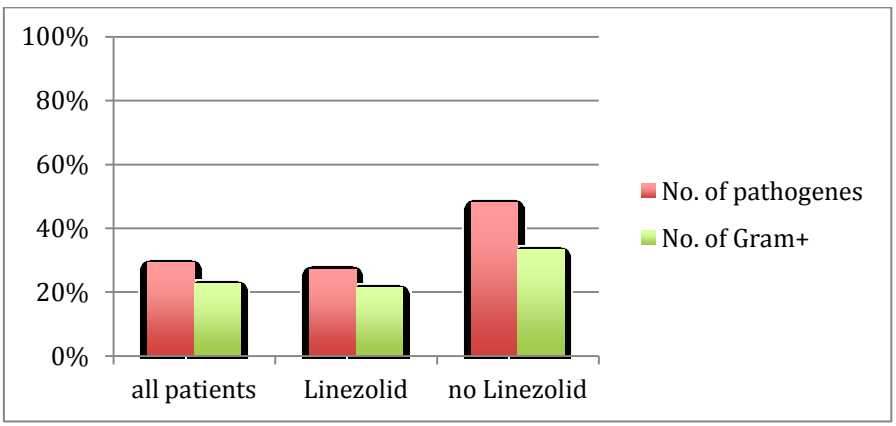

Figure 7 Number of pathogenes/Gram+ in bloodcultures

Next, a total of 1410 routine surveillance cultures of nasal, throat, genital and rectal swabs were analysed. In the linezolid group 1064 samples were taken (median 37) and, out of these, 346 (32.5%) displayed bacterial outgrowth (median 8.5 per patient). In 202 cases the identified bacteria were positive for Gram staining (20%) and in nine swabs from three different patients a linezolid resistant enterococcus faecium was found. In the no linezolid- group 200 swabs were taken (median 28) and 61 (30.5%) pathogens were found. Again the majority were Gram positive bacteria (n= 49; 24.5%, see also figure). The patient, in whom a positive blood culture displayed a Linezolid-resistant enterococcus faecium, had four surveillance swabs with an outgrowth of this linezolid resistant enterococcus.

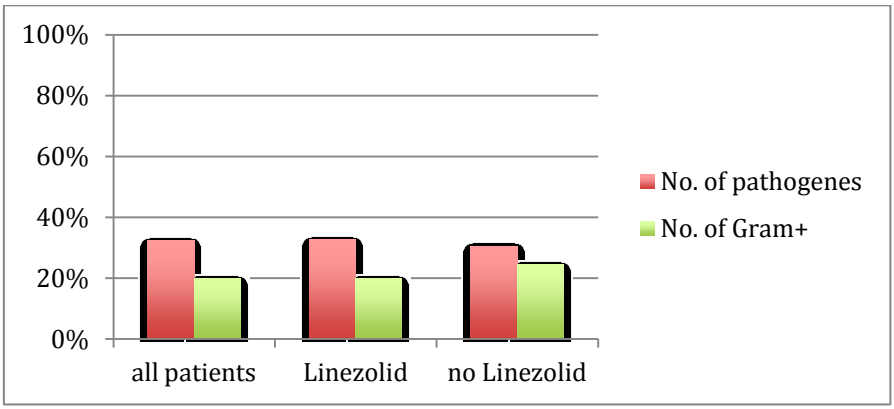

Figure 8 Number of pathogenes/Gram+ in swabs

A total number of 444 routine surveillance stool cultures were taken in the whole cohort. In the linezolid group 225 out of 387 samples (58.1%) were positive for bacterial outgrowth including 99 cases with Gram positive bacteria (25.6%).Among these, no isolate was found to be linezolid-resistant. In the no linezolid group 23 out of 57 stool cultures (40.4%) were positive including 17 Gram positive bacteria (29.8%). Again, the patient, in whom a positive blood culture displayed a linezolid-resistant Enterococcus faecium, had two stool cultures with an outgrowth of this linezolid resistant enterococcus

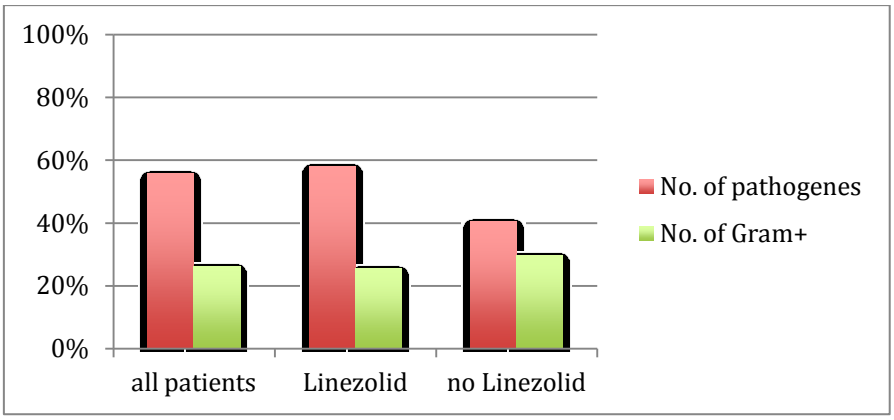

Figure 9 Number of pathogenes/Gram+ in stool cultures

Finally, 382 other cultures including urine cultures were analyzed. In the linezolid group 41 out of 344 samples (11.9%) were positive with 13 (3.7%) displaying Gram positive bacteria. No resistance to linezolid was found. In the no linezolid group 42 other cultures were taken, but no pathogens were found.

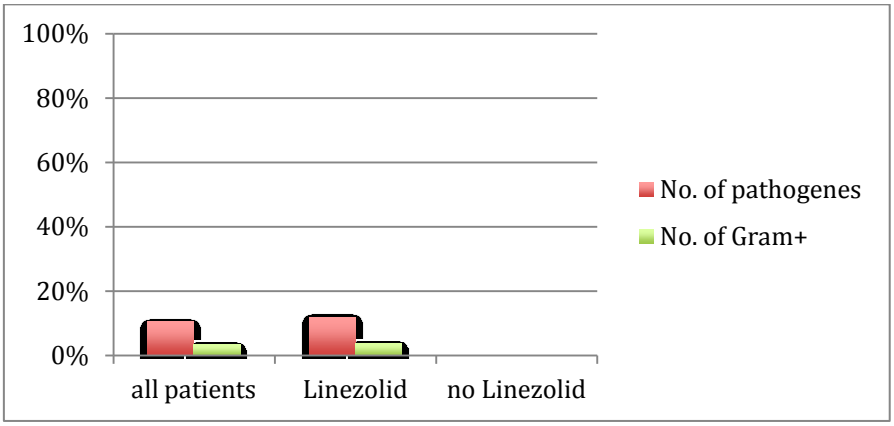

Figure 10 Number of pathogenes/Gram+ in other cultures

In summary, in three out of 28 patients (10.7%) undergoing UCB-SCT a linezolid-resistant Enterococcus faecium emerged during linezolid treatment for febrile neutropenia. These resistant Enterococci were identified during routine surveillance cultures of nasal, throat, genital and rectal swabs.

	collective n=35	Linezolid n=28	no Linezolid n=7
fever>38° (d)			
Median	6	6	3
average	7,78	8,55	4,29
Range	0 - 28	0 - 28	0 - 12
number of bloodcultures	**266**	**239**	**27**
Median	6	8	4
average	7,19	8,0	3,86
range	1 - 16	1 - 16	1 - 10
No. of pathogenes	**78 29,32%**	**65 27,20%**	**13 48,15%**
median	2	1,5	2,5
average	2,11	2,17	1,86
range	0 - 11	0 - 11	0- 4
No. of Gram+	**60 22,56%**	**51 21,34%**	**9 33,33%**
median	1	1	0
average	1,62	1,7	1,29
Range	0- 9	0- 9	0-4
number of swabs	**1264**	**1064**	**200**
Median	34	37	28
average	34,16	35,47	28,57
range	0 - 94	0 - 94	11 - 38
No. of pathogenes	**407 32,20%**	**346 32,52%**	**61 30,50%**
median	9	8,5	10
average	11	11,53	8,71
range	0- 43	0- 43	2- 14
No. of Gram+	**251 19,86%**	**202 18,98%**	**49 24,50%**
median	5	4	6
average	6,78	6,73	7
range	0- 29	0- 29	1- 13
number of stool cultures	**444**	**387**	**57**
Median	11	12	9
average	12	12,9	8,14
range	0- 49	0- 49	0- 15
No. of pathogenes	**248 55,86%**	**225 58,14%**	**23 40,35%**
median	6	6,5	3
average	6,70	7,50	3,29
range	0- 39	0- 39	0- 8
No. of Gram+	**116 26,13%**	**99 25,58%**	**17 29,82%**
median	2	1	2
average	3,14	3,30	2,43
range	0- 20	0- 20	0- 6
number of other cultures	**386**	**344**	**42**
Median	9	10	6
average	10,43	11,47	6
range	1- 29	1- 29	2- 12
No. of pathogenes	**41 10,62%**	**41 11,92%**	**0 0%**
median	0	0,5	0
average	1,11	1,37	0
range	0- 8	0- 8	0- 0
No. of Gram+	**13 3,37%**	**13 3,78%**	**0 0%**
median	0	0	0
average	0,35	0,43	0
range	0- 3	0- 3	0- 0

Table 6 Number of pathogens/Gram + in cultures and swabs

4 Discussion

Linezolid is an antibiotic approved for the treatment of infections caused by otherwise resistant Gram-positive bacteria, like VRE as well as MRSA, and frequently used in the setting of HSCT. (26) , Among critically ill and neutropenic patients with cancer VRE is one of the most common causes of blood stream infections and associated with a considerable high rate of mortality (47). ‘80 percent of patients with acute leukemia develop fever during chemotherapy induced neutropenia (34) and more than 70 percent of treatment associated deaths are the result of an infection.’ (35,36) Thus, the right anti-infective therapy in the setting of HSCT is inevitable. Even though prospective studies in patients undergoing HSCT are missing, linezolid is a welcomed and often-used drug, in need to treat HSCT patients with Gram-positive bacteremia.
Although well tolerated in the general population there are remaining concerns about possible myelosuppressive side effects and reports of resistant bacteria. (37,38) ‘Drug-induced myelosuppression, a well-documented side effect of many antibiotics and antiviral drugs, is relevant when treating patients undergoing hematopoietic cell transplantation.’ (48) However, the impact of linezolid on hematopoietic cell recovery after UCB-HSCT has not been described sufficiently.

In this study we examined 35 patients who underwent UCB-HSCT, among them 28 patients having received linezolid, in order to find out the impact of linezolid on neutrophil and platelet engraftment and to determine emerging resistance to linezolid. We found out that resistance to linezolid occurred in some cases, but hematopoietic recovery after UCB-HSCT was not affected.

4.1 Treatment of infections with resistant gram positive bacteria

From 2000-2005 linezolid was the only available drug for treatment of VRE and among few drugs for treatment of other infections with resistant Gram-positive bacteria. In the recent years, alternatives with activity against VRE and other resistant Gram-positive bacteria have emerged: daptomycin (Cubicin®), tigecycline (Tygacil®) or quinupristin/dalfopristin (Synercid®). However, for the time being linezolid and quinupristin/dalfopristin are the only antibiotics approved for VRE treatment by the Food and Drug Administration (FDA). (28)

Tigecycline (TGC) is a glycylcycline used for the treatment of complicated skin and soft tissue infections as well as complicated intraabdominal infections. It shows an expanded spectrum of activity, including multidrug resistant species like MRSA, VRE, and extended-spectrum-betalactamase-producing Enterobacteriaceae (ESBLE). (39) In a study conducted recently from K.S Schwab et al. (39) TGC showed good response rates and low toxicity rates in patients treated for febrile neutropenia. Unfortunately TGC achieves low serum concentrations, hence the effect on bloodstream infections is questioned. (40) Furthermore it is not approved for hospital-acquired pneumonia. Another study of Giampaolo et al showed good results of TGC combined with piperacillin/tazobactam in febrile neutropenic hematologic patients. (41). However, in a case report of 2013 describing two boys undergoing HSCT and treatment with tigecycline concerns about possible myelotoxicity were raised. (48)

Daptomycin is indicated in complicated skin infections (cSSSI) caused by e.g. Staphylococcus aureus including MRSA, and Enterococcus faecalis (but only vancomycin-sensitive). It is not effective in treatment for pneumonia, so the use is restriced in critically ill patients. (42) Resistance to VRE has been reported.

Quinupristin/dalfopristin is indicated in cSSSI caused by Staphylococcus aureus including MRSA, and VRE resistant Enterococcus faecium. Unfortunately, activity against Enterococcus faecalis is lacking and Gram-positive super-infections have been reported. (44) In addition, the application is restricted also because of venous irritations, mild to severe arthralgias and myalgias. (43)

VRE has become increasingly common in neutropenic patients in general, but also in the setting of UCB-HSCT. Most infections are caused by Enterococcus faecium and a smaller number by E. faecalis, E. avium, and other kinds. (45) Since 1/3 of patients is colonised with VRE in the gastrointestinal tract, it is suggested that high dose chemotherapy leads to damage of the mucosa and VRE can access circulation consequently. (45) Another hypothesis was mentioned in a study from Kamboj et al (47): also antibiotics like ciprofloxacin can lead to high colonisation of VRE in the gut by breaking down intact immune defense mechanisms. Finally, the use of vancomycin itself can contribute to VRE. 1/3 of the patients may proceed to bacteremia and another third died of bloodstream infection. (45) Several studies report the association between VRE infections and very high mortality. Avery et al. (46) conducted a study between 1997 and 2003 and found a 100% mortality of 12 allogeneic HSCT patients having a VRE infection. Another retrospective study between 2008 and 2009 at the Memorial Sloan-Kettering Cancer Center (MSKCC) reported a much higher incidence of VRE bloodstream infections as mentioned in previous studies. 23 out of 43 patients (53.5%) who underwent allogeneic HSCT had VRE found in the blood cultures and in 9% death was linked to VRE infection.(47) However, it is still unclear. if VRE infection is the main cause of death or just a marker of poor prognosis. Linezolid and daptomycin are used as a salvage therapy, but it is still not known if the treatment is sufficient and can reduce the mortality rate.

In this study resistance to linezolid occurred in three out of 28 patients undergoing UCB-HSCT. In all cases the isolated pathogen was Enterococcus faecium, which was identified in nine out of 1064 swabs from three different patients. These findings are in good accordance with rising rates of resistances, which have been reported especially after prolonged use of linezolid. In a case report from Dibo et al. (31) a linezolid resistant Enterococcus faecialis was identified in a UCB

recipient. Gonzales et al (55) collected data of 45 patients treated with linezolid and found one resistant pathogen. Another study reported on the isolation of linezolid-resistant, vancomycin-resistant Enterococcus faecium in seven patients after liver, kidney, and pancreas transplantation. (56) Rahim et al. (57) even reported two cases of linezolid- and vancomycin-resistant Enterococcus faecium (LRVREF) prior linezolid exposure presumably transmitted nosocomially.

Therefore it is suggested that linezolid should be used carefully and may be tested on VRE prior to application followed by tight control measures of infection. (56,57)

4.2 Umbilical cord blood stem cell transplantation in hematologic malignancies

As an alternative stem cell source UCB currently accounts approximately for up to 20% of all allogeneic HSCT. Used since 1993, HLA matched UCB considerably helps to find more suitable donors, especially for minority populations, and shortens the search process. Primarily used in children it became widespread in the adult population using often two or more grafts. Still UCB is linked with slow immune recovery and graft failure compared to bone marrow transplantation. (49,51) Neutropenia is a major limitation based on the small number of HSC and progenitor cells infused. (54)

However more and more studies have published improving results with UCB-HSCT. Petterson et al. (49) were collecting data of 135 paediatric patients between 1995-2005. ‘Neutrophil engraftment occurred in 83% of patients by day 42 (median 23 days) and platelet engraftment in 55% by day 60 (median 56 days). CD34 cell doses over 1.7 x 10^5/kg body weight promoted faster engraftment.’ (49). Also other studies reported the value of nucleated cell dose for hematopoietic recovery. (53) In our study population, neutrophil engraftment occurred in 81.1 % of patients by day 33, platelet engraftment occurred in 75.7% of patients by day 35 supporting and confirming results of other studies. However, a recent study conducted by Le Bourgeois et al. (50) compared, the hematopoietic recovery of patients, who received peripheral blood stem cells, with patients recieving double

UCB. They found a similar percentage of engraftment although the median time to platelet recovery was significantly higher in the dUCB group (38 days vs 0 days). Wagner et al (51) reported similar engraftment rates after MUD-HSCT and UCB-HSCT in 102 patients. Also other studies conclude that UCB from unrelated donors can sufficiently restore hematopoiesis in adults. Later engraftment depends on the fact that patients receiving UCB only get 1/10 as many nucleated cells as recipients of adult HSC. (52) Hence it is considered if an HLA matched unrelated donor is not available and the transplantation is needed, UCB can be used in the treatment of adults with hematologic malignancies (54). Our results are in line with these studies and compare well to current guidelines in UCB transplantation. The median $CD34^+$ cell dose infused was 1.54x10^5/kg body weight in our study and four patients received a double unit cord blood graft. The median time to neutrophil and platelet recovery was on day 33 and day 35.5, respectively. 30 out of 35 patients were able to reach ANC500 and 28 out of 35 patients reached PLT20.

On first sight the overall survival does not look promising due to a 3-year survival of 31% implying that only 1 in 3 patients survived. However, it needs to be taken in consideration that these patients were without any chance to get cured with another type of therapy. In a study of Laughlin et al. (52) the overall survival in recipients of UCB, MMUD and MUD bone marrow was compared. The results did not show any difference between recipients of mismatched marrow and cord blood (20%, 26% survival after 22 months, respectively) and a higher overall survival in patients receiving matched bone marrow (35%). 26 percent overall survival in UCB recipients is in very good accordance to that reported in our study.

4.3 Myelosuppressive effects of antibiotics after stem cell transplantation

Due to prolonged periods of profound neutropenia and lower rates of neutrophil as well as platelet recovery patients undergoing UCB-HSCT are at a high risk for death related to infections. (52) Although several studies addressed the role of antibiotic treatment on hematopoietic recovery after UCB-HSCT, our study is the first one investigating the impact of linezolid treatment on neutrophil and platelet recovery in this setting. In a retrospective case-controlled study from Cohen et al. (28) conducted from 2000 until 2007 in patients undergoing allogeneic HSCT with a MRD or MUD, no differences in the times to neutrophil and platelet engraftment were noted in patients receiving either linezolid or vancomycin. `Times to ANC500 and PLT20 were similar in the cases and controls, as were the times to ANC1000 and PLT50` (28). However, they claimed that larger studies with prolonged neutropenia and linezolid use for more than14 days are needed. Due to the stem cell source used in their study (either bone marrow or mobilized peripheral blood stem cells of adult donors) the median time to neutrophil engraftment was 12 days, which is relatively fast as compared to median 33 days in our study. The median duration of linezolid therapy was 14 days compared to 16 days in our study. In a randomized, double blind study by Jaksic et al. (29) linezolid and vancomycin were compared in febrile neutropenic patients with cancer after chemotherapy. There were no differences in platelet counts between both treatment groups, but a transient delay in absolute neutrophil recovery not affecting the mean duration of treatment in patients receiving linezolid. In a third study from Smith et al. (9) linezolid was compared to other antibiotic agents in patients with cancer and neutropenia. The study did not reveal any differences in blood counts in patients with neutropenia and compassionate use of linezolid. In summary, none of the published studies could show an increased risk of hematologic adverse effects in cancer patients' recieving linezolid treatment. (30)

The data of our retrospective analysis therefore confirm and expand previous observations on the safety of linezolid use on hematologic engraftment. Linezolid was well tolerated in UCB-HSCT patients, neutrophil and platelet engraftment was similar in the two groups and only few linezolid resistant pathogens were found.

Despite concerns about possible myelosuppression, even in the setting of prolonged neutropenia after UCB-HSCT and linezolid treatment for a median of 16 days we could not find any adverse effect on engraftment in our retrospective analysis.

4.4 Limitations of the study

This study has several limitations. First it is a retrospective analysis, and a prospective analysis is definitely warranted to determine the effect of linezolid on platelet and neutrophil engraftment. Secondly, the control group was low in numbers due to the fact that only 7 patients received no linezolid; thirdly, in general the sample size of our study cohort with only 35 patients was rather small. These relatively low patient numbers did not allow elucidating subtle effect of linezolid on hematopoietic recovery. Even though there were several limitations, the quality of collected data was good, and we could confirm the safety of linezolid in highly vulnerable patients in terms of hematopoietic recovery.

4.5 Conclusion

We found that linezolid had no negative impact on neutrophil and platelet engraftment in patients having undergone UCB-HSCT. Hence it is suggested that linezolid can be administered safely in such patients. Therefore, in the absence of prospective, randomized clinical trials, these data support the use of linezolid in allogeneic HSCT including UCB-HSCT for treatment of adults in agreement with published studies. However, the occurrence of linezolid resistant gram- positive Enterococci may be a matter of concern. Close microbial monitoring is therefore warranted in patients treated with linezolid. Larger prospective and randomized studies are eventually needed to establish the definitive role of linezolid treatment in UCB-HSCT patients.

5 References

1. Edward A, Copelan, MD. Hematopoietic Stem-Cell Transplantation. New England Journal of Medicine 2006;354:1813-26.

2. Donnall E, Lochte HL, Cannon JH, Sahler OD, Ferrebee JW. Supra lethal whole body irradiation and isologous marrow transplantation in man. The journal of clinical investigation 1956 June 19.

3. Devetten M, Armitage JO. Hematopoietic cell transplantation: progress and obstacles. Annals of Oncology 2007;18:1450-1456.

4. Apperley J, Carreras E, Gluckman E, Masszi T. The EBMT Handbook, Haematopoietic Stem Cell Transplantation. 6th ed. Paris: forumservice; 2012.

5. Buchholz S, Ganser A. Hämatopoetische Stammzellentransplantation: Indikationen,Grundlagen und Ausblick. Internist 2009;50:572-580.

6. Ballen KK, KorethJ,Chen Y, Dey BR, Spitzer TR. Selection of optimal alternative graft source: mismatched unrelated donor, umbilical cord blood, or haploidentical transplant. Blood 2012;119:1972-1980.

7. Horn M. Biochemie des Menschen. 5th ed. Germany: Georg Thieme Verlag KG; 2002.

8. Apfalter P, Gastl G, GeisslerD, Girschikofsky M, Kahls P, Keil F,et al. Febrile Neutropenie. Österreichische Ärzte Zeitung 2012 Apr.

9. Smith PF, Birmingham MC, Meagher AK et al. Safety, efficacy and pharmacokinetics of linezolid for treatment of Gram-positive infections in cancer patients with neutropenia. Annals of Oncology 2003;14(5):795-801.

10. Adulte Stammzellen. Hoffnungsträger der modernen Medizin. Available from:http://www.medunigraz.at/images/content/file/presse/folder/folder_stammzellen.pdf. Accessed June 30, 2014.

11. Berger DP, Engelhardt R, Mertelsmann R. Das rote Buch: Hämatologie und internistische Onkologie. 5th ed. Heidelberg, München, Landsberg,Frechen, Hamburg: Hühtig Jehle Rehm GmbH; 2014.

12. Michl M.Basics:Hämatologie.3rd ed. München: Elsevier GmbH; 2013.

13. Eisenmann A, Fischer U, Kozyga K, Nepp B, Priebe B, Schleicher B et al. Transplant Jahresbericht 2012. Stubenring 6, 1010 Wien: Gesundheit Österreich GmbH; 2012.

14. Passweg JR, Baldomero H, Peters C, Gaspar HB, Cesaro S, Dreger P et al. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. Bone Marrow Transplantation 2014;49:744–750.

15. European society for Blood and Marrow transplantation. Available from:http://www.ebmt.org/Contents/Resources/Library/Patientanddonorpublications/Documents/12.%20NMDPIntroBooklet.pdf. Accessed January 27, 2014.

16. De Bock R, Middelheim AZ. Febrile neutropenia in allogeneic transplantation. International Journal of Antimicrobial Agents 2000;16:177–180.

17. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T. 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clinical infectious diseases 2002;34:730-51.

18. Picazo JJ. Management of the febrile neutropenic patient: A consensus conference. Clinical infectious diseases 2004;39(1):1-6.

19. Castagnola E, Faraci M. Management of bacteremia in patients undergoing hematopoietic stem cell transplantation. Expert review of anti-infective Therapie 2009;7(5):607-621.

20. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II et al. Executive Summary: Clinical Practice Guideline for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer: 2010 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases 2011;52(4):427–431.

21. Swaney SM, Aoki H, Ganoza C, ShinabargerDL.The Oxazolidinone Linezolid Inhibits Initiation of Protein Synthesis in Bacteria. Antimicrobial Agents and Chemotherapy 1998;42(12):3251.

22. Whitehouse T, Cepeda JA, Shulman R, Aarons L, Nalda-Molina R, Tobin C, MacGowan A, Shaw S et al. Pharmacokinetic studies of linezolid and teicoplanin in the critically ill. Journal of Antimicrobial Chemotherapy 2005 March;55(3):333-340.

23. French G. Safety and tolerability of linezolid. Journal of Antimicrobial Chemotherapy 2003;51(2):45-53.

24. Shaw KJ, Barbachyn MR. The oxazolidinones: past, present, and future. Annals of the New York Academy of Sciences 2011;1241:48–70.

25. Dryden MS. Linezolid pharmacokinetics and pharmacodynamics in clinical treatment. Journal of Antimicrobial Chemotherapy 2011;66(4):iv7–iv15.

26. Pfizer. 2014.Zyvox (linezolid) official website for healthcare professionals. Available from: http://www.pfizerpro.com/hcp/zyvox. Accessed February 4th, 2014.

27. Lawrence KR, Adra M, Gillman K. Serotonin Toxicity Associated with the Use of Linezolid: A Review of Postmarketing Data. Clinical Infectious Diseases 2006;42(11):1578-1583.

28. Cohen N, Mihu CN, Seo SK, Chung D, Chou J, Heller G, et al. Hematologic Safety Profile of Linezolid in the Early Periengraftment Period after Allogeneic Stem Cell Transplantation. Biology of Blood and Marrow Transplantation 2009;15:1337-1341.

29. Jaksic B, Martinelli G, Perez-Oteyza J, Hartman CS, Leonard LB, Tack KJ. Efficacy and Safety of Linezolid Compared with Vancomycin in a Randomized, Double-Blind Study of Febrile Neutropenic Patients with Cancer. Clinical Infectious Diseases 2006;42:597–607.

30. Gould KF. Linezolid: safety and efficacy in special populations. Journal of Antimicrobial Chemotherapy 2011;66(4):iv3–iv6.

31. Dibo I, Pillai SK, Gold HS, Baer MR, Wetzler M, Slack JL, et al. Linezolid-Resistant Enterococcus faecalis Isolated from a Cord Blood Transplant Recipient. Journal of Clinical Microbiology 2004 April;42(4):1843–1845.

32. Legout L, Senneville E, Gomel JJ et al. Linezolid-induced neuropathy. Clinical Infectious Diseases 2004;38:767-8.

33. Apodaca AA, Rakita RM. Linezolid-induced lacticacidosis. New England Journal of Medicine 2003;348:86-7.

34. Glasmacher A, Lilienfeld-Toal M, Schulte S, Hahn C, Schmidt-Wolf IG, Prentice A. An evidence-based evaluation of important aspects of empirical antibiotic therapy in febrile neutropenic patients. Clinical Microbiology and Infection 2005;11(5):17–23.

35. Schiel X, Link H, Maschmeyer G, Glass B, Cornely OA, Buch- heidt D, Wilhelm M, Silling G, Helmerking M, Hiddemann W, Ostermann H, Hentrich M. A prospective, randomized multi- center trial of the empirical addition of antifungal therapy for febrile neutropenic cancer patients: results of the Paul Ehrlich Society for Chemotherapy (PEG) Multicenter Trial II. Infection 2006;34:118–26.

36. Hiddemann W, Maschmeyer G, Link H, Helmerking M, Adam D. Therapy of infections in patients with acute leukemia. Med Klin (Munich) 1997;92:406–9.

37. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplantation 2005;36:757–769.

38. Gonzales RD, Schreck PC, Graham MB, Kelkar S, DenBesten K, Quinn JP. Infections due to vancomycin-resistant Enterococcus faecium resistant to linezolid. The Lancet 2001 April 14:357(9263):1179.s

39. Schwab KS, Hahn-Ast C, Heinz WJ, Germing U, Egerer G, Glasmacher A, Leyendecker C, Marklein G et al. Tigecycline in febrile neutropenic patients with haematological malignancies: a retrospective case documentation in four university hospitals. Infection 2014;42:97–104.

40. Anthony KB, Fishman NO, Linkin DR, Gasink LB, Edelstein PH, Lautenbach E. Clinical and Microbiological Outcomes of Serious Infections

with Multidrug-Resistant Gram-Negative Organisms Treated with Tigecycline. Clinical Infectious Diseases 2008;46(4):567-570.

41. Bucaneve G, Micozzi A, Picardi M, Ballanti S, Cascavilla N, Salutari P et al. Result of multicenter, controlled, randomized clinical trial evaluating the combination of Piperacillin/Tazobactam and Tygecycline in high risk hematologic patients with cancer with febrile neutropenia. Journal of Clinical Oncology 2014;32.

42. Cubicin package insert. Lexington, MA: Cubist Pharmaceuticals, Inc;2013 available from: http://cubicin.com/pdf/PrescribingInformation.pdf. Accessed May 16, 2014.

43. SYNERCID (quinupristin and dalfopristin) injection, powder, lyophilized, for solution [Pfizer Laboratories Div Pfizer Inc]. Available from: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=28f698df-b584-4455-2e94-6efc41313960. Accessed May 16, 2014.

44. Linden PK, Moellering RC Jr, Wood CA et al. Treatment of vancomycin resistant Enterococcus faecium infections with quinupristin/dalfopristin. Clinical Infectious Diseases 2001;33:1816-1823.

45. Weinstock DM, Conlon M, Iovino C, Aubrey T, Gudiol Carlota, Riedel E et al. Colonization, Bloodstream Infection, and Mortality Caused by Vancomycin-Resistant Enterococcus Early after Allogeneic Hematopoietic Stem Cell Transplant. Biology of Blood and Marrow Transplantation 2007 May;13(5):615-621.

46. Avery R, Kalaycio M, Pohlman B, et al. Early vancomycin-resistant enterococcus (VRE) bacteremia after allogeneic bone marrow transplantation is associated with a rapidly deteriorating clinical course. Bone Marrow Transplantation 2005;35:497–499.

47. Kamboj M, Chung D, Seo SK, Pamer EG, Sepkowitz KA, Jakubowski AA, et al. The changing Epidemiology of Vancomycin-resistant enterococcus (VRE) bacteremia in allogeneic hematopoietic stem cell transplant (HSCT) recipients. Biology of Blood and Marrow Transplantation 2010 Nov; 16(11): 1576-1581.

48. Maximova N, Zanon D, Pharm D, Verzegnassi F, MD, Granzotto M. Neutrophils Engraftment Delay During Tigecycline Treatment in 2 Bone Marrow–transplanted Patients. Journal of Pediatric Hematology/Oncology 2013;35:e33–e37.

49. Petterson TE, Gabriel M, Tiedemann K, Teague L, Shaw PJ, Baker D et al. Outcome following unrelated cord blood transplant in 136 patients with malignant and non-malignant diseases: a report from the Australian and New Zealand children's haematology and oncology group. Bone Marrow Transplantation 2009;43:207-215.

50. Le Bourgeois A, Mohr C, Guillaume T, Delaunay J, Malard F, Loirat M et al. Comparison of Outcomes after Two Standards-of-Care Reduced-Intensity Conditioning Regimens and Two Different Graft Sources for Allogeneic Stem Cell Transplantation in Adults with Hematologic Diseases: A Single-Center Analysis. Biology of Blood and Marrow Transplantation 2013;19:934-939.

51. Wagner JE, Barker JN, DeFoer TE, Baker KS, Blazar BR, Eide C et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and non malignant diseases: influence of CD34 cell dose and

HLA disparity on treatment-related mortality and survival. Blood 2002 Sep 1;100(5).

52. Laughlin MJ, M.D, Barker J, M.D, Bambach B, M.D et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. New England Journal of Medicine 2001 June 14;344(24).

53. Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R et al. Outcome of cord blood transplantation from related and unrelated donors. New England Journal of Medicine 1997;337:373-381.

54. Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. The Lancet Oncology 2010;11:653-60.

55. Gonzales RD, Schreckenberger PC, Graham MB, Kelkar S, DenBesten K, Quinn JP. Infections due to vancomycin-resistant Enterococcus faecium resistant to linezolid. The Lancet 2001;357:1179.

56. Herrero IA, Issa NC, Patel R. Nosocomial spread of linezolid-resistant, vancomycin-resistant. Enterococcus faecium. New England Journal of Medicine 2002;346:867–869.

57. Rahim S, Pillai SK, Gold HS, Venkataraman L, Inglima K, Press RA. Linezolid-resistant, vancomycin-resistant Enterococcus faecium infection in patients without prior exposure to linezolid. Clinical Infectious Diseases 2003;36:146-148.