DIPLOMA THESIS

Retrospective evaluation of pretreatment prognostic indicators in patients with lymphoma after autologous or allogeneic stem cell transplantation

submitted by

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For the Academic Degree of

Doktorin der gesamten Heilkunde

(Dr.^{in} med. univ.)

at the

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Graz, March 30th 2017

Statutory Declaration

I hereby formally declare that I have written the submitted thesis independently and without any outside support except for the quoted literature and other sources mentioned in the paper. I clearly marked and separately listed all of the literature and all of the other sources which I employed when producing this academic work, either literally or in content.

Graz, March 30th 2017 *Veronika Zach eh.*

Danksagung

Zuallererst möchte ich mich bei meinen beiden Betreuern Frau Dr.in med. univ. Dr.in scient.med. Katharina Prochazka und Herrn ao. Univ.-Prof. Dr.med univ. Peter Neumeister für die Bereitstellung dieses spannenden Themas und ihre Unterstützung bei der Fertigstellung dieser Arbeit bedanken. Katharina hatte immer ein offenes Ohr für mich und reagierte stets prompt und effizient auf jede noch so kleine Frage. Beide standen mir in entscheidenden Phasen unterstützend zur Seite und erleichterten mir so die Vollendung dieser Arbeit.

Besonders möchte ich mich auch bei meinen Eltern und meinen beiden Brüdern bedanken – für ihren Rückhalt, ihre bedingungslose Unterstützung, ihre Zeit und ihren Zuspruch, die mich schon mein ganzes Leben lang begleiten. Meine Eltern sind die großzügigsten Menschen, die ich kenne und sind in so vielerlei Hinsicht Vorbild und Antrieb für mich. Unzählbare Male haben sie mich in Gesprächen ermutigt und bestärkt und sind mir mit ihrer Erfahrung und Lebensweisheit zur Seite gestanden. *„Der Weg entsteht im Gehen“* – diese Worte wird mein Vater nicht müde zu wiederholen, wenn ich vor so mancher, anfangs so groß erscheinender Aufgabe - wie auch dieser Arbeit - stehe, und jedes Mal ermutigen sie mich, die Herausforderung anzunehmen.

Zu guter Letzt möchte ich mich bei meinen Freunden und Freundinnen bedanken, die mich teils schon seit dem Kindergarten und der Schule begleiten und mir immer mit Rat und Tat und aufmunternden Worten zur Seite stehen.

Zusammenfassung

Hintergrund: Mit mehr als 100.000 Neudiagnosen pro Jahr weltweit zählt das Multiple Myelom (MM) zu den häufigsten malignen Erkrankungen des hämatologischen Formenkreises. Der Forschungfortschritt der letzten Jahre führte zur Entwicklung einer Vielzahl neuer Therapieansätze. In Anbetracht all dieser neuen therapeutischen Möglichkeiten, ist es nötig die Bedeutung der Stammzelltransplantation in der Behandlung des MM neu zu evaluieren. Die autologe sowie die allogene Stammzelltransplantation bergen immer noch eine Vielzahl großer Risiken für den/die Patienten/in, und verlässliche prognostische Faktoren, die den weiteren Verlauf nach der Transplantation vorhersagen bzw. beeinflussen, sind noch nicht ausreichend identifiziert und analysiert. Der ideale Parameter für einen solchen Zweck sollte vor der Transplantation leicht und schnell zu messen und überwachen sein. Diese Studie hat es sich zum Ziel gesetzt solche PatientInnencharakteristika und Laborparameter zu finden und auf ihre Aussagekraft hinsichtlich progressionsfreiem Überleben und Gesamtüberleben zu überprüfen.

Material und Methoden: Diese retrospektive Studie basiert auf einem Kollektiv von 232 PatientInnen, die mit einem MM diagnostiziert wurden und sich zwischen 2004 und 2015 am Landeskrankenhaus Graz und am Landeskrankenhaus Hochsteiermark einer Stammzelltransplantation unterzogen. Über 30 verschiedene PatientInnencharakteristika und Laborparameter zum Zeitpunkt der Aufnahme zur Transplantation, die im MEDOC System dokumentiert waren, wurden erhoben und hinsichtlich ihres Einflusses auf das progressionsfreie Überleben und Gesamtüberleben der PatientInnen analysiert. Die statistische Auswertung, bestehend aus deskriptiver Datenanalyse, univariater Cox Regression und Kaplan-Meier Kurven, wurde mit EXCEL 2010 und SPSS 23 durchgeführt.

Ergebnisse: In der statistischen Analyse gelang es, jeweils einen Zusammenhang zwischen niedrigen Hämoglobinwerten (<12g/dl vs ≥12g/dl, p=0,0238), einer verminderten Thrombozytenzahl (<100 G/l vs ≥100 G/l, p<0,0001) und einer geringen Lymphozyten/Monozyten Ratio (<1,2 vs ≥1,2, p=0,0452) und vermindertem Gesamtüberleben festzustellen. Außerdem konnte ein erhöhtes CRP, anhand von unvariater Cox Regression und Kaplan-Meier Kurven, als ein unabhängiger Faktor für längeres progressionfreies Überleben identifiziert werden (≤5mg/l vs >5mg/l, p=0,0262).

Conclusio: In dieser Arbeit haben wir gezeigt, dass die vor der Transplantation erhobenen Parameter Hämoglobin, Thrombozytenzahl, Lymphozyten/Monozyten Ratio und CRP den weiteren Verlauf nach einer Stammzelltransplantation bei MM entscheidend beeinflussen können. Nach weiterer Bestätigung dieser Ergebnisse in einer größeren, prospektiven Studie, könnte es möglich werden, das Outcome vieler PatientInnen durch - sofern klinisch umsetzbar - entsprechende Adaption dieser Parameter vor der Transplantation, zu verbessern.

Abstract

Background: With more than 100,000 newly diagnosed cases each year, Multiple Myeloma (MM) ranks among the most common types of blood cancers. In the last decade, increased research led to the development of a multitude of new treatment strategies. All these new and promising therapeutic approaches resulted in the need of a reevaluation of the role of stem cell transplantation in myeloma patients. Autologous and allogeneic stem cell transplantation are still considered high-risk procedures and reliable prognostic indicators for inferior/ superior outcome after the transplantation have not been detected and analysed sufficiently. The ideal prognostic parameter should be easy, fast and cheap to measure and monitor before the transplantation. The aim of this retrospective study was to identify patient characteristics and laboratory parameters that influence Progression Free Survival (PFS) and Overall Survival (OS).

Material and Methods: This retrospective study is based on a population of 232 patients diagnosed with MM and treated with stem cell transplantation at the Landeskrankenhaus Graz und Landeskrankenhaus Hochsteiermark between 2004 and 2015. Over 30 different patient characteristics and laboratory parameters at the date of admission for stem cell transplantation documented in the MEDOC System were collected and analysed with regard to their influence on PFS and OS. The statistical evaluation, including descriptive data analysis, univariate Cox Hazards Models and Kaplan-Meier estimators, was performed using EXCEL 2010 and SPSS 23.

Results: The statistical evaluation revealed decreased haemoglobin levels (<12 g/dl vs ≥ 12 g/dl, $p=0.0238$), reduced thrombocyte levels (<100 G/l vs ≥ 100 G/l, $p<0.0001$) and a low lymphocyte/monocyte ratio (<1.2 vs ≥ 1.2, $p=0.0452$) as factors associated with inferior OS. Furthermore, univariate Cox Hazard Models and Kaplan-Meier estimators identified elevated CRP as an independent prognostic indicator for superior PFS (≤ 5mg/l vs >5mg/l, $p=0.0262$).

Conclusion: In this study we demonstrated that pre-transplantation haemoglobin, thrombocyte count, lymphocyte/monocyte ratio and CRP influence the further course of patients after stem cell transplantation in MM. After the confirmation of these results in a larger, prospective study the next step could include improving the outcome of many MM patients after stem cell transplantation by – as far as possible in an acute, clinical setting – adjusting these parameters before the patient undergoes transplantation.

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List of Abbreviations

MH	Morbus Hodgkin
NHL	Non-Hodgkin Lymphoma
WHO	World Health Organization
PTLD	Posttransplantlymphoproliferative disorders
B-NHL	B-cell Non-Hodgkin Lymphoma
T-NHL	T-cell Non-Hodgkin Lymphoma
DLBCL	Diffuse large B-cell Lymphoma
FL	Follicular Lymphoma
EBV	Epstein-Barr Virus
HHV 8	Human Herpesvirus 8
HIV	Human Immunodeficiency Virus
HTLV	Human T-lymphotropic Virus
HCV	Hepatitis C Virus
MALT-Lymphoma	Mucosa-associated lymphoid tissue – Lymphoma
etc.	et cetera
IPI	International Prognostic Index
LDH	Lactate dehydrogenase
CR	complete response
OS	overall survival
ECOG	Eastern Cooperative Oncology Group
MM	Multiple Myeloma
vs	versus
IMWG	International Myeloma Working Group
CRAB	increased **c**alcium, **r**enal dysfunction, **a**naemia, **b**one lesions
CT	computed tomography

PET	positron emission tomography
MDE	Myeloma defining event
MRI	magnetic resonance imaging
e.g.	example given
f.i.	for instance
IgH	immunoglobulin heavy chain
ESR	erythrocyte sedimentation rate
HVS	hyperviscosity syndrome
CRP	C-reactive protein
IgG	immunoglobulin Type G
IgA	immunoglobulin Type A
IgE	immunoglobulin Type E
IgD	immunoglobulin Type D
M-spike	monoclonal Ig spike
FDG-PET	fludeoxyglucose - positron emission tomography
(R-)ISS	(Revised-) International Staging System
CA	chromosomal aberration
MGUS	Monoclonal Gammopathy of Undetermined Significance
SM	Smoldering Myeloma
DNA	Deoxyribonucleic acid
VTD	**V**elcade® (bortezomib), **t**halidomide, **d**examethasone
CDT	**c**yclophosphamide, **d**examethasone, **t**halidomide
VRD	**V**elcade® (bortezomib), **R**evlimid® (lenalidomide), **d**examethasone
RD	**R**evlimid® (lenalidomide), **d**examethasone
ASCT	autologous stem cell transplantation

G-CSF	granulocyte colony stimulating factor
MP-scheme	melphalan, prednisolone
MPT	melphalan, prednisolone, thalidomide
MPV	melphalan, prednisolone, velcade® (bortezomib)
ESA	erythropoiesis-stimulating agent
LKH	Landeskrankenhaus
TX	transplantation
d0	day 0 = day of transplantation
HCT-CI	haematopoietic cell transplant-comorbidity index
HLA-match	human leukocyte antigen-match
ANC	absolute neutrophil count
T-regeneration	thrombocyte - regeneration
ANC-regeneration	neutrophil – regeneration
GvHD	Graft-versus-Host Disease
SPSS	Statistical Package for Social Sciences
BMI	Body Mass Index
VL	Various lymphoma
MCL	Mantle Cell Lymphoma
CLL	Chronic Lymphocytic Leukemia
CNS	central nervous system
PCL	Plasma Cell Leukemia
sibl.	sibling
MUD	matched unrelated donor
UCB	umbilical cord blood
BEAM	**c**armustine (**B**CNU), **e**toposide, cytarabine (**A**raC), melphalan
BCNU	bis-chloroethylnitrosourea
AraC	cytosine arabinosid e

BeEAM	**be**ndamustine, **e**toposide, cytarabine (**A**raC), **m**elphalan
PR	partial remission
VGPR	very good partial remission
PD	progressive disease
SD	stable disease
RIC	reduced-intensity conditioning
MAC	myeloablative conditioning
CMV	cytomegalovirus
LMR	lymphocyte/monocyte ratio
PFS	Progression free survival
FU	follow up
EFS	event free survival
ALC	absolute lymphocyte count

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1 INTRODUCTION

1.1 Lymphoma

When talking about Lymphoma, Hodgkin Lymphoma, a disease with very special pathological characteristics and the possibility of very specific treatment, has to be distinguished from Non-Hodgkin Lymphoma (NHL), making up all other forms of this complex neoplastic entity. (1)

Besides the exceptional position of Hodgkin Lymphoma, plasma cell neoplasms, which will be a major part of this thesis, are also considered special in the large field of lymphomas. Unlike classic NHL, they typically derive from bone marrow and only rarely affect the peripheral blood and lymph nodes. (1)

In early 2016, the World Health Organization (WHO) reached a new consensus among haematopathologists, clinicians and geneticists and revised the *Classification of Lymphoid Neoplasms* published in 2008. (2)

The 2016 classification takes clinical, genotypic, morphologic and immunophenotypic characteristics into account and provides the following scheme, dividing lymphomas considering the original cell into:

1) Mature B-cell neoplasms
2) Mature T and Natural Killer neoplasms
3) Hodgkin Lymphoma
4) Posttransplantlymphoproliferative disorders (PTLD)
5) Histiocytic and dendritic cell neoplasms (2)

1.1.1. Non-Hodgkin Lymphoma

1.1.1.1 Definition

NHL derives from the neoplastic transformation of the primary effector cells of the immune system. They either come from the B-cell-system (B-NHL) or the T-cell-system (T-NHL). (3)

Looking at growth and dissemination habits, NHL, can be divided into two different types:

- Low grade (indolent) NHL, growing and spreading rather slowly, and
- High grade (aggressive) NHL, which are likely to grow and spread very fast (4)

High grade lymphomas occur, with 3-5 cases/100,000 people/year (3), about 3 to 4 times more often than low grade lymphomas. (5)

1.1.1.2 Epidemiology

In western industrialised countries, incidence rates have been rising within the last years. (6) Statistik Austria has kept track of NHL incidences in Austria from 1983 to 2012. Ranging from 492 newly diagnosed cases of NHL in 1983 to 1,265 in 2012, this increase in incidence rates can also be observed in Austria. (7) As a reason for this significant rise, increased life expectancy with its associated higher risk of neoplasms is discussed. (6)

Worldwide about 15-25 new cases/100,000 people, with a slight emphasis on male, are reported. (3)

In general NHL can strike at any age but the risk of development of this disease gets higher, the older a person gets. The average age of onset is slightly over 60 years. (3)

The most common subtypes of NHL are diffuse large B-cell lymphoma (DLBCL) with about 31%, and follicular lymphoma (FL) with about 22%. (5,8) T-NHL only accounts for about 5-10% of all NHL. (8)

1.1.1.3 Pathogenesis

Several aetiologic and moleculargenetic factors are discussed to play a role in the development of NHL. Aetiologic features involved in the genesis of NHL, include viral and bacterial infection, congenital and acquired immunodeficiency syndromes, ionising radiation, mutagenic substances and environmental and hereditary factors. (3)

Infections and immune suppression are to be especially emphasised here. Epstein-Barr virus (EBV), Human herpesvirus 8 (HHV 8), Human immunodeficiency virus (HIV), Human T-lymphotropic virus (HTLV) and Hepatitis C virus (HCV) are all known to play a role in the pathogenesis of NHL. (5) Infection with EBV for example, is often associated with endemic Burkitt Lymphoma in Africa and Asia. (3) In bacterial infections, Helicobacter pylori and Chlamydia psittaci have to be highlighted, since they raise the risk of developing a mucosa-associated lymphoid tissue – lymphoma (MALT-lymphoma). (3,5)

Patients who receive immunosuppressive therapy or who are suffering from immunodeficiency syndromes, are at higher risk of lymphoma. For HIV-positive patients e.g. the risk of developing lymphoma is 60 times higher than for patients who are HIV negative. (5)

Furthermore the risk of lymphoma is increased by several autoimmune diseases, the two most important being Sjögren syndrome and systematic lupus erythematosus. (5)

1.1.1.4 Clinical Presentation

The clinical presentation often depends on the anatomic structures affected by the disease. About two thirds of NHL present as nodal enlargement with cervical, axillar, paraaortal and inguinal lymph nodes being the main sites. (1,6)

In the other third of NHL, symptoms caused by extranodal manifestations in skin, central nervous system, gastrointestinal tract etc. make up the main signs of presentation. (1,6) These signs of extranodal manifestations can include spleno- and hepatomegaly, fatigue, loss of efficiency, increased susceptibility to infections and tendency to bleed, anaemic pallor and several more. (3)

Lymphadenopathy and presentation of extranodal manifestation are often accompanied by the so-called „B-symptoms“ – general symptoms including unexplained weight-loss, exceeding 10% of a patient’s normal weight over the last six months, night sweat and fever 38°C and higher. (5,6) These B-symptoms occur more often in low-grade than in high-grade NHL. (5)

1.1.1.5 Staging and Grading

In order to choose the most adequate therapy to treat and potentially even cure NHL, it is indispensable to perform a meticulous staging evaluation. (9)

When a patient is newly diagnosed with NHL, the tumour is first and foremost histologically classified according to the WHO classification. (9)

In the second step, clinical staging is performed and the patient is assigned a stage using the Ann Arbor classification (Table 1). General clinical staging evaluation starts with precise medical history taking and thorough clinical examination. In addition, the patient needs to undergo several further investigations such as detailed laboratory tests including a complete blood count, specific blood chemistry parameters and parameters indicating liver and renal function, image-taking of abdomen, chest and pelvis and a bone marrow aspiration and biopsy. (5,9,10)

With the help of the findings of these examinations, the Ann Arbor staging is applied. This staging system, initially designed for the evaluation of Hodgkin's Lymphoma, is now the commonly used system for the anatomic staging of NHL. (9,10)

In the Ann Arbor staging system, NHL are divided into 4 principal stages, depending on localisation related to the diaphragm and expansion. (10)

Principal stages	
I	Involvement of one lymph node or extranodal organ or site (IE)
II	Involvement of ≥ 2 lymph node regions on the same side of the diaphragm, or localised involvement of an extranodal site or organ (IIE) and one or more lymph node regions on the same side of the diaphragm
III	Nodal sites on both sides of the diaphragm involved, might be accompanied by localised involvement of an extranodal organ or site (IIIE) or spleen (IIIS) or both (IIISE)
IV	Diffuse or disseminated involvement of one or more distant extranodal organs with or without associated lymph node involvement
Modifiers	
A	Absence of B-symptoms
B	Presence of B-symptoms

Table 1: Ann Arbor staging system for NHL **(10)**

In a third step the patient should be assigned to a risk group, using the International Prognostic Index (IPI) (Table 2). (9)

The IPI was established as a result of a study including 2,031 patients with aggressive NHL treated with a doxorubicin-based combination chemotherapy regimen. In this study, five pretreatment factors turned out to be independently of value in the prediction of a patient‘s outcome. These five factors are namely the patient‘s age, the Ann Arbor Stage, the number of extranodal sites involved, the patient‘s performance status and the serum level of lactate dehydrogenase (LDH). (10,11)

Based on their score of risk factors, patients can be divided into 4 different risk groups: low risk (0 or 1), low-intermediate risk (2), high intermediate risk (3) and high risk (4 or 5) (Table 3). Patients assigned to the low risk group, tend to have a significantly better outcome concerning CR- (complete response) and OS-(overall survival) rates than those assigned to the high risk group. (10,11)

Criteria	**+**		**-**
Age	≤ 60 years		> 60 years
Ann Arbor stage	I or II (localized)		III or IV (advanced)
Number of extranodal sites involved	0-1	**vs**	≥ 2
Patient's performance status	ECOG 0 or 1		≥ 2
LDH level (serum)	Normal		abnormal

Table 2: International Prognostic Index (IPI) **(6,10,11)**

Risk group	**IPI score**	**CR Rate**	**5-year survival**
Low	0 or 1 point	87%	73%
Low-intermediate	2 points	67%	50%
High-intermediate	3 points	55%	43%
High	4 or 5 points	44%	26%

Table 3: International Prognostic Index (IPI) scores and outcome **(6,11)**

1.1.2 Multiple Myeloma

1.1.2.1 Definition

Multiple Myeloma (MM), historically referred to as Morbus Kahler, is a clonal type of B-cell-lymphoma, characterized by an accumulation of monoclonal plasma cells in the bone marrow. These plasma cells are producing an increased amount of fragmented or complete immunoglobulins, so-called paraproteins, which can be detected in the patients‘ serum and / or urin. (8,12)

In 2015 the International Myeloma Working Group (IMWG) revised the old criteria for the diagnosis of MM. Before this update, there had to be evidence of an end-organ manifestation to make the diagnosis of a MM. The presence of end-organ damage was known as the so-called CRAB criteria, containing increased **c**alcium levels, **r**enal dysfunction, **a**naemia and destructive **b**one lesions. For the new definition of active myeloma three additional markers were established. If one of these “myeloma defining events” (MDE) - namely ≥ 60% plasma cells in the bone marrow or serum involved / uninvolved free light chain ratio ≥ 100 or >1 focal MRI lesion ≥5mm - is present in a patient, his / her risk of suffering myeloma-caused organ damage in the following two years is about 80%. (13)

The new definition of active MM is visualised in the following table (Table 4):

2015 IMWG definition of active multiple myeloma:

Clonal bone marrow plasma cells ≥10%
or
Biopsy-proven bony or extramedullary plasmacytoma

and
*any **one or more** of the following CRAB features and myeloma-defining events:*

- *Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:*
    - *Hypercalcaemia: serum calcium >0.25 mmol/L (>1mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11mg/dL)*
    - *Renal insufficiency: creatinine clearance <40 mL per minute or serum creatinine >177µmol/L (>2mg/dL)*
    - *Anaemia: haemoglobin value of >20g/L below the lowest limit of normal, or a haemoglobin value <100g/L*
    - *Bone lesions: one or more osteolytic lesion on skeletal radiography, CT, or PET/CT. If bone marrow has <10% clonal plasma cells, more than one bone lesion is required to distinguish from solitary plasmacytoma with minimal marrow involvement*

- *Any one or more of the following biomarkers of malignancy (MDEs):*
    - *60% or greater clonal plasma cells on bone marrow examination*
    - *Serum involved / uninvolved free light chain ratio of 100 or greater, provided the absolute level of the involved light chain is at least 100mg/L (a patient's "involved" free light chain—either kappa or lambda—is the one that is above the normal reference range; the "uninvolved" free light chain is the one that is typically in, or below, the normal range)*
    - *More than one focal lesion on MRI that is at least 5mm or greater in size.*

Table 4: IMWG definition of active myeloma **(13)**

MM has to be distinguished from asymptomatic or smoldering myeloma, presenting with similar laboratory signs but without clinical manifestation in end-organs. (12) Smoldering myeloma can potentially transform into MM. In this case, this previously mostly no therapy requiring neoplasm needs to be treated sufficiently. (12,14)

1.1.2.2 Epidemiology

With about 5.6/100,000 cases per year, MM is a comparatively common disease in the broad field of haematologic neoplasms. (3,5) In patients older than 60 years, the incidence rate rises to 8/100,000 cases per year. (3) Only 2% of all cases occur under the age of 40. (12) The median age of onset lies at 71 years for men and 73 years for women. (8)

Some sources suggest, that with a ratio of 3:2 men are slightly more often affected than women, (3) others report equal distribution in sexes. (5)

Among black people, incidence rates for MGUS, which often predates MM, are twice as high as among whites. A large study published in *Blood* in 2010 came up with new results concerning age of onset and survival. This study, conducted on 5,798 blacks and 28,939 whites, suffering from MM, found a younger age of onset but superior survival in the first years in blacks. White individuals though tend to have better survival chances over longer time. (15)

The lowest incidence rates for MM can be found in China. (8)

1.1.2.3 Aetiology and Pathogenesis

Aetiologic and pathogenetic aspects are to some extent still unknown. Several potential risk factors such as chronic infections, exposition to ionising radiation, a history of organ transplantation and pernicious anaemia are discussed to play a role in the development of MM. Contact with pesticides and chemicals e.g. used in metal or wood processing industries could also increase the risk of Myeloma.

(3,5,8) Further obesity and familial predisposition are believed to be of significance. (3,8)

Recently two main pathogenetic pathways are assumed to account for the emergence of MM. (16,17)

The first theory suggests that immunoglobulin heavy chain (IgH) translocations lead to a dysregulation and / or overexpression of oncogenes. (12,16,17) Affected oncogenes are - in almost all MMs - mainly cyclin D1, D2 or D3. (17) IgH translocations can be found in about 50% of all myeloma patients. Recurrent affected loci are 11q13, 4p16, 6p21, 16q23 and 20q11. In about 10-20% of all cases, immunoglobulin light chain translocations can be found. (16,17)

Hyperdiploidy of chromosomes, also leading to the overexpression of type D cyclins, lies in the focus of the second pathway discussed. This feature appears in about 50% of MM. (16,17)

Cyclin D dysregulation is an early and common sign, occuring in about two thirds of all MM. (17) Late alterations include point mutations, deletions, epigenetic irregularities and secondary translocations. (12)

MM is characterised by an accumulation of clonal terminally differentiated MM cells in the bone marrow. (16) MM cells can be described as special types of plasma cells, which stand out because of their longevity and relatively low proliferation rate. (18)

MM cells interact with the surrounding cells in and around the bone marrow, leading to several restructuring processes concerning bone remodelling, angiogenesis and cell immunity. (16) The MM cells‘ influence on vicinal cells provides them with support and protection from apoptosis on the one hand. On the other hand it explains some of the typical clinical symptoms like hypercalcaemia and bone lesions. (16,19)

1.1.2.4 Diagnosis

The diagnosis of MM is based on a complex combination of anamnesis, clinical examination, laboratory tests and instrumentational diagnostics. (20)

Clinical presentation

About 15-20% of all patients are diagnosed with MM in the context of a routine checkup. In about 80% of all cases of asymptomatic patients, the first finding leading to the diagnosis is an increased erythrocyte sedimentation rate (ESR). (16)

Common signs and symptoms at the time of diagnosis include ostealgia and pathological fractures, anaemia, recurrent bacterial infections, central nervous symptoms, peripheral neuropathy, uraemia, fever and several more (Table 5). (16)

Ostealgia and pathological bone fractures are the most frequent findings in patients at the time of diagnosis. In many cases, the bone pain is located along the spine. Osteolysis in this region can lead to vertebral collapse and even a compression of the cord in the worst case. Other typical fractures occur in the long hollow bones. Bone lesions in the skull can be seen as hypointense areas in the x-ray. This specific appearance is usually referred to by the term „pepperpot skull“. (5,16)

The second most important symptom in newly diagnosed patients is anaemia. Most frequently it presents itself normochromic and normocytic and comes with typical epiphenomenons like fatigue, lassitude and pallor. (12,16)

Furthermore, an increased vulnerability to bacterial and viral infections can be observed. This arises due to the secondary malfunction of immunoglobulins and neutropenia. (12,16)

Renal failure is mostly caused by the so-called cast-nephropathy, induced by bence-jones-proteins clogging the renal tubular system. (16) The increased bone loss results in hypercalcaemia. Classic symptoms of renal failure and hypercalcaemia are polydipsia, polyuria, nausea, vomiting, amentia and ultimately oliguria and anuresis. (3,12,16)

Other common features include abnormal bleeding tendency, due to incomplete function of platelets and coagulation factors and thrombocytopenia, amyloidosis and hyperviscosity syndrome. (12,16)

Amyloidosis is caused by the deposition of amyloid in several organs, including heart, kidney, gastrointestinal tract and lungs. Amyloid originates from monoclonal plasma cells producing immunoglobulin light chains. Amyloidosis is an independent disease but due to the fact that it arises from some of the same chromosome translocations, it can be found as a complication in about 10% of myeloma patients. (3,21) The deposition of these light chains leads to skin lesions, oedema, changes in the constitution of the tongue, diarrhea, cardiac failure and many other symptoms. (12,21)

In later stages of the disease, about 2% of MM patients show signs of a hyperviscosity syndrome (HVS). (3,12) Bleedings of mucosa and skin and / or central nervous symptoms including vertigo, dizziness, headache and visual and auditory dysfunctions are suspicious for the existence of HVS. (12,16,22)

Clinical signs and symptoms at the time of diagnosis (in 1700 patients at the University of Heidelberg)	
Clinical Sign or Symptom	**Patients (%)**
Bone pain and pathological fractures	65
Anaemia	48
Severe bacterial infection	5
Central nervous symptoms	3
Peripheral neuropathy	3
Uraemia	3
Increased bleeding tendency	3
Herpes zoster	2
Fever as a B symptom	1
Oedema	1
Diagnosed in the context of routine checkup	25

Table 5: Clinical signs and symptoms at the time of MM diagnosis **(16)**

Laboratory results

- **Complete blood count** and **white blood cell differential, total protein, kidney values, calcium, LDH, C-reactive protein (CRP), electrolytes (20)**: Frequent findings include normochromic and normocytic anaemia and rouleaux formation in the smear, high total protein as well as neutropenia, thrombocytopenia and low levels of serum albumin in advanced stages. In about 45% of cases, serum calcium values are increased. An elevation of serum creatinine values can be found in about 20% of patients. (3,12)
- **Immunoglobulin electrophoresis** (Figure 1) of serum and urin to discover **paraprotein:** In about 60% of patients this paraprotein is type G immunoglobulin (IgG), furthermore, immunoglobulin A (IgA) and light chain only paraproteins are found quite frequently. Findings of immunoglobulin E (IgE) or immunoglobulin D (IgD) paraprotein are

rare. (12) Monoclonal Igs usually appear as conspicuous „M (=monoclonal)-spike“ in electrophoresis while serum albumin levels are low (Figure 2). (1) Serum electrophoresis of MM of the Bence-Jones protein type does not show this specific M-peak but rather presents itself with a decrease in the γ-globulin fraction and low albumin values. (16)

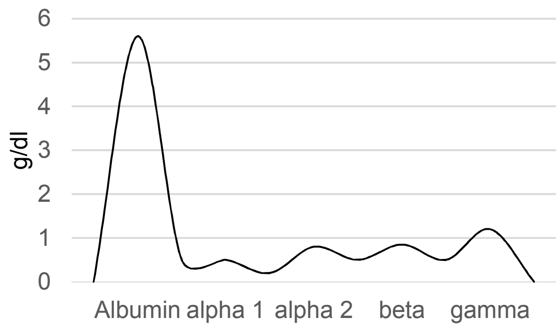

Figure 1: Normal serum protein electrophoresis

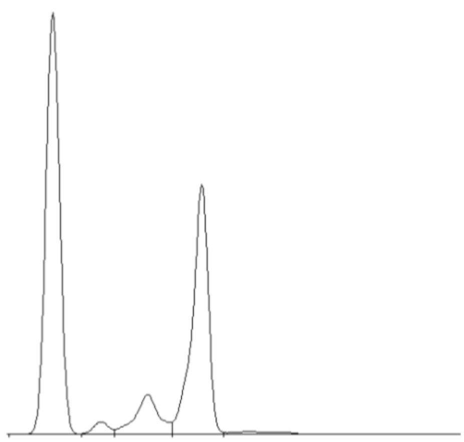

Figure 2: Serum protein electrophoresis of a patient with MM: Increased Total Protein, M-spike in the β-globulin region, decreased levels of Albumin and γ-globulin.

- **Immunoglobulin-free light chains:** Plasma cells produce κ and λ light chain proteins, which are not paired with heavy chains. Usually only few of these immunoglobulin-free light chains are synthesised. If the plasma cells are malignantly degenerated, a rise in the serum value of κ or λ free light chains can be observed. The best way to detect a serum elevation of immunoglobulin-free light chains is with a free light chain assay. (12)
- **Immunoparesis and Bence-Jones proteinuria:** Low normal immunoglobulin values are a common finding in many MM patients. This characteristic is usually referred to as immunoparesis. Urinary paraproteins, so-called Bence-Jones proteins, can be found in about 66% of all cases. (12) Light chains are normally filtered and reabsorbed in the kidney. In case of overproduction, as it occurs in MM, this filtration / reabsorption balance cannot be kept, resulting in Bence-Jones proteinuria. (23)
- increased **ESR** (12,20)
- **Serum beta-2-microglobulin:** As many studies in the last years suggest, beta-2-microglobulin values are discussed to serve as markers of tumour burden (24) as well as survival (25) and progression in asymptomatic myeloma. (12,26) Increased beta-2-microglobulin levels can also be found in patient's with impaired kidney function and in a variety of other diseases including liver disorders and several different neoplasms. Due to this lack of specifity, this parameter should be interpreted with caution. (8,27)

Bone marrow diagnostics

Bone marrow examination is an indispensable part of attaining the diagnosis of MM. The bone marrow of MM patients usually shows often abnormal plasma cells making up over 20-30% of the marrow. (1,12)

The morphology of these plasma cells varies from normal looking plasma cells over plasma blasts to distinct atypical cells. Really big cells, diffuse cell margins, multiple cell nuclei, an increased nucleus / cytoplasmic ratio, a variable amount of nucleoli and several features more are signs that indicate malignant transformation. (6) So-called flame cells, named after their red cytoplasm (Figure 3), Mott cells, characterized by Ig-globes in their cytoplasm, giving them their honeycombed appearance and cells with variable inclusions (Russell or Dutcher bodies) (Figure 4) are other frequent findings in MM bone marrow. The higher the stage, the higher the probability that not only the bone marrow is affected by MM but also other organs like spleen, lungs, kidney, liver, lymph nodes etc.. (1)

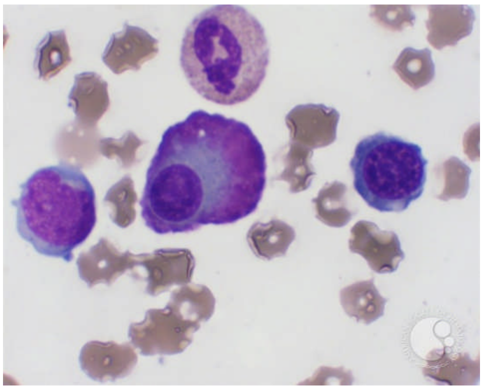

Figure 3: Flame cell, mostly found in IgA MM, with typical red cytoplasmatic inclusions **(28,29)**

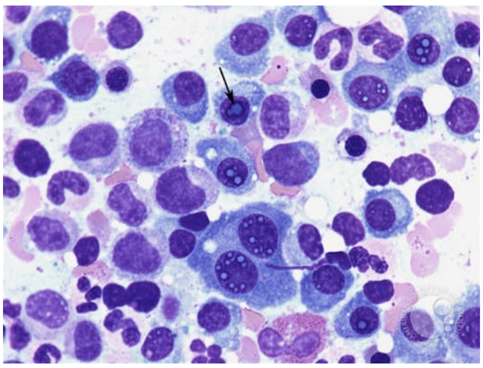

Figure 4: MM cells with inclusions, so-called Dutcher bodies (arrow), in the nucleus **(30)**

The histology reveals different growth patterns. The early disease is characterized by focal or interstitial growth, while advanced MM grows diffusely, eliminating normal haematopoiesis. The percentage of plasma cells can ascend up to 100%. (6)

Malignant plasma cells tend to feature a specific immunophenotype, typically CD38high, CD138high and CD45low. (12)

Instrumental diagnostic procedures

A whole body low-dose computed tomography (CT) -scan has replaced the x-ray as the method of choice to determine the extent of the bone affection in MM. (8)

Magnetic resonance imaging (MRI) is a good way to detect extramedullary manifestations and assess the total tumour burden. (8,31) When neurological symptoms occur, it should be implemented f.i. to detect a compression of the spinal cord. (8)

In addition, an echocardiography should be performed to rule out cardiac amyloidosis and to assess the heart function prior to the use of anthracyclins and high-dose chemotherapy. (8,20)

Often fludeoxyglucose-positron emission tomography (FDG-PET) can be useful to evaluate extramedullary manifestation and as an early attempt to predict a patient’s response to therapy. (8,31,32) However, due to the fact that its role has not yet been sufficiently proven especially in prospective studies, FDG-PET is not used routinely in the diagnostics of MM. (8)

1.1.2.5 Staging

The 1975 Salmon Durie classification taking into account haemoglobin, calcium, bone constitution, paraprotein and serum creatinine, made the tumour burden its main focus. This staging system, consisting of three stages, was used for a long time. Due to the heterogenity of the patients especially in stage three, it is not possible to draw conclusions concerning a patient's prognosis. (8)

In 2005, with the International Staging System (ISS) (Table 6), another attempt towards creating a proper internationally workable staging system was made. The ISS only examines two simple parameters: beta-2-microglobulin and albumin. On the basis of these values it divides patients into three groups and allows an estimation of the median survival time. (8,33)

International Staging System (ISS)		
Stage	**Concentration in serum**	**Median survival time**
I	β2-microglobulin < 3.5 mg/l and albumin ≥ 3.5 g/dl	62 months
II	neither stage I nor stage III	44 months
III	β2-microglobulin ≥ 5.5 mg/l	29 months

Table 6: International Staging System (ISS) using beta-2-microglobulin and albumin to allow an estimation of the survival time in MM **(33)**

In 2015 the IMWG revised the ISS adding chromosomal aberrations (CA) and serum LDH as new parameters to the ISS (Table 7). To test for CA, an interphase fluorescent in situ hybridization should be performed. High-risk CAs, including del(17p), t(4;14) and several more, are differentiated from standard-risk CAs. The prognosis for patients with high-risk CAs is worse than that for patients with a standard-risk type of myeloma. (34)

A similar effect can be detected when looking at LDH. High levels of LDH are associated with more aggressive courses of the disease and poorer outcome. (34)

Revised International Staging System (R-ISS)				
Revised Group	**ISS-Stage**	**Criteria**	**5-year OS**	**5-year PFS**
R-ISS I	ISS stage I	no high-risk CA [del(17p) and/or t(4;14) and/or t(14;16)] *and* normal LDH level (less than upper limit of normal range)	82%	55%
R-ISS II	all other possible combinations		62%	36%
R-ISS III	ISS stage III	High-risk CA or High LDH-level	40%	24%

Table 7: Revised International Staging System (R-ISS) improving the ISS by adding chromosomal aberrations and LDH as important prognostic parameters **(34)**

1.1.2.6 Therapy

Before starting treatment, the diagnosis of MM has to be confirmed and distinguished from Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Myeloma (SM). (5)

The revised IMWG criteria for the diagnosis of MM, mentioned above, made treatment now not only accessible for patients complying with one or more of the CRAB features (hyper**c**alcaemia, **r**enal insufficiency, **a**naemia, **b**one lesions), but also for patients presenting with any one or more MDE (≥ 60% plasma cells in the bone marrow, serum involved / uninvolved free light chain ratio ≥ 100, >1 focal MRI lesion ≥5mm). (13,35)

Myeloma treatment consists of specific and supportive strategies. (12)

Specific treatment

An adequate therapy is chosen in consideration of a patient‘s age and the comorbidities. The aim of the therapy is to reach remission and fast symptom relief while simultaneously avoiding severe side effects. Furthermore, it is indispensable to take into account the patient‘s individual social and mental situation. (8)

There are two different treatment strategies:

- intensive combination chemotherapy
- non-intensive therapy (12)

Intensive therapy

Intensive therapy is mostly used for patients under the age of 70. (12) However, this age limit can be applied quite flexibly. Patients older than 70 years, f.i., who do not have any major comorbidities, can also be eligible for this kind of treatment. (8)

Intensive therapy starts with four to six cycles of chemotherapy, consisting of different combinations of bortezomib (**V**elcade®), lenalidomide (**R**evlimid®), **t**halidomide, **d**examethasone or **c**yclophosphamide. (12) Bortezomib acts via the inhibition of proteasomes. (36) Lenalidomide and thalidomide are considered immunomodulatory agents, which also have an antiproliferative, antiangiogenetic and apoptotic effect. The steroid dexamethasone augments the response to the other agents while at the same time working against side effects of cytostatic therapy. (36) Cyclophosphamide is an alkylating agent, preventing the tumour’s DNA synthesis. (37)

Frequently used schemes are VTD (bortezomib, thalidomide and dexamethasone), CDT (cyclophosphamide, dexamethasone and thalidomide), VRD (bortezomib, lenalidomide and dexamethasone) or RD (lenalidomide and dexamethasone). The substances can be administered orally or intravenously. (12)

Induction therapy is followed by stem cell apheresis, high-dose chemotherapy and autologous stem cell transplantation (ASCT). (12)

To mobilise the stem cells and cause their leachate into peripheral blood after polychemotherapy, a haematopoietic growth factor like granulocyte colony stimulating factor (G-CSF) is applied. It is necessary to collect at least 2 x 10^6 - better 5 x 10^6 - CD34-positive cells/kg body weight. (8)

Before the transplantation, the patient has to undergo a myeloablative conditioning therapy with high-dose melphalan (200mg/m2 body surface for patients ≤ 70 years, 140 mg/m2 body surface for patients > 70 years (3)). Thereafter the collected and cryopreserved stem cells get defrosted and reimplanted. (8) In some cases a second ASCT needs to be performed. (8,12)

If patients are treated with intensive therapy, a significant improvement of median life expectancy can be observed. However, still only 10% of these patients eventually get cured. (12)

Allogeneic stem cell transplantation is discussed to be a potential way of curing the disease. (3,8,12) Since this procedure comes with severe side effects, it can only be applied to treat few, highly selected patients. (12)

New promising studies suggest good results with so-called tandem-transplantations where an allogeneic transplantation is performed after an ASCT. (8)

Non-intensive therapy

For decades, the best way to treat older, non transplantable patients, was the combination of melphalan and prednisolone the so-called „Alexanian-Schema“ (MP-Scheme). Nowadays this scheme - slightly modified and augmented with new substances – is still in use. (8)

Approved therapy models include melphalan and prednisolon combined with thalidomide (MPT) or bortezomib (MPV). (5) A study from 2013 revealed, that after a median follow-up of about 5 years, a patient’s risk of dying was 31% lower for

MPV than for MP. (38) Another study from 2007 shows a significantly improved overall survival for patients treated with MPT instead of MP. Median overall survival times after a median follow-up of slightly more than 4 years were 51.6 months for MPT and 33.2 months for MP. (39)

Non-intensive chemotherapy is mostly administered every 4-6 weeks (3) for a maximum of 18 months, (12) sometimes even longer. Positive effects on paraprotein values, bone constitution and blood composition can be observed after the treatment. (12)

Treatment of relapses

Recurrence of typical symptoms and an increase in characteristic blood parameters are indications of a relapse. (12) Relapse in MM is nearly inescapable. With each relapse, treatment gets harder and the time to the next relapse shortens and shortens until the myeloma reaches a refractory state. (40) Protocols for the treatment of relapses are composed of the substances mentioned above and new therapeutical components like monoclonal antibodies. (5)

Consolidation and maintenance are often in the center of attention of studies trying to overcome the nearly inevitable relapse. Completely new agents, immunotherapy, special strategies directly tailored to individual patients, MM vaccines or the complex inhibition of myeloma immune checkpoints could be the future of myeloma therapy. (40)

Supportive care

First of all, the patient needs to be stabilised and urgent attendant symptoms have to be taken care of. (5) Due to conglomerations of paraprotein, the function of the kidney is often hindered. Therefore it is important that patients drink enough and never get dehydrated to prevent further clogging. In the case of renal failure, the focus is on rehydration, treatment of hyperuricaemia and hypercalcaemia and kidney substituting programmes like dialysis. (12)

Bone alterations, including osteoporosis, osteolysis and in some cases osteosclerosis, can present themselves as significant pain and pathologic fractures resulting in a loss of quality of life. (35)

Bone lesions and their symptoms can be treated with bisphosphonates, which prevent further progression and can also normalise calcium levels in case of hypercalcaemia. (12,35) Zolendronate and pamidronate are potent agents for this special indication. (35)

Complex fractures or compression of the spinal cord often require surgical intervention. (16,35) Violent bone pain can be reduced by local radiotherapy. (35)

Therapeutic options for the treatment of anaemia include Erythropoiesis-stimulating agents (ESAs) and red blood cell transfusions. (35) Patients receiving chemotherapy with haemoglobin values lower than 10g/dl are thought to benefit most from the administration of ESAs. Patients with haemoglobin levels lower than 8 g/dl or those who cannot undergo ESA therapy because of its risks or who do not respond to ESA therapy, should receive red blood cell transfusions. Transfusions should also urgently be applied to patients who suffer from severe symptoms of anaemia and need fast relieve. (35)

Another great danger derives from the increased vulnerability to infections. Noteworthy pathogens include pneumococci, haemophilus influenzae and viral infections, f.i. Influenza A and B. Patients, close family members as well as health personnel should undergo vaccinations against these frequent agents. In some cases, a prophylaxis with antiviral compounds, antibiotics or immunoglobulins is recommended. (35)

Other potentially necessary supportive measures involve plasmapheresis to treat hyperviscosity syndrome and the use of different schemes to handle severe pain. (5,35) Pain management can consist of radiotherapy, surgical approaches, analgesics administered by the WHO pain ladder, adjuvants like antidepressants or anticonvulsants and co-medications like anti-emetics. (35)

2 MATERIAL AND METHODS

2.1 Parameters of evaluation

For this study, the data of a total of 396 patients, who underwent stem cell transplantation (TX) at the Landeskrankenhaus Graz (LKH Graz) and at the Landeskrankenhaus Hochsteiermark (LKH Hochsteiermark) between January 1st 2004 and March 17th 2015, were collected.

Where available, up to 36 parameters were extracted from the patient records in the Open MEDOC system.

General parameters taken down for every patient were gender, date of birth, body mass index and diagnosis.

Furthermore, several details about the patient's therapy were collected, including the kind of TX that was performed, the conditioning therapy he / she received, whether he / she received reduced-intensity or myeloablative conditioning and the day of the TX (d0). In addition the patient's pre-TX status and his / her haematopoietic cell transplant-comorbidity index (HCT-CI) were noted down in the chart.

Where patients underwent allogeneic stem cell transplantation, the donor's gender, the cytomegalovirus status of patient and donor, and the human leukocyte antigen-match (HLA-match) were evaluated.

Laboratory parameters collected included values for leukocytes, the absolute neutrophil count (ANC), lymphocytes, monocytes, haemoglobin, thrombocytes, LDH, creatinine, bilirubin, protein, albumin and CRP. All laboratory parameters were withdrawn from blood tests taken on the patients's admission day to the hospital for the TX.

Post-transplantation parameters gathered from the patients' health records were the time until T-regeneration (thrombocytes >20,000), the time until ANC-regeneration (ANC >500), the date of discharge, the amount of days a patient stayed in the hospital after the TX, the occurrence of a relapse, the date of a relapse, the patient’s status (dead, alive), the date of the last follow up and whether or not a graft versus host disease (GvHD) emerged and if yes, which organ system was affected (allogeneic transplanted patients only).

2.2 Basic evaluation and statistical analysis

The work on this thesis started with the establishment of a database in Microsoft Excel version 2010 using the Open MEDOCS of the LKH Graz as a datasource.

For the statistical analysis, two different programmes were used. The descriptive statistics were calculated using Microsoft Excel 2010, while the Statistical Package for Social Sciences version 23.0 (SPSS inc., Chicago, IL,USA) was used for the performance of the explorative statistics.

The bar charts, histograms and tables were also created with Microsoft Excel.

Once all the data were collected and properly taken down in an extensive chart, some general descriptive calculations were performed, to create a first overview of the database. These calculations included f.i. a look at the distribution of genders and the different entities of disease etc..

For further evaluation, it was decided to concentrate only on the 232 cases of MM. A detailed descriptive analysis of this data set was conducted in Microsoft Excel.

Afterwards SPSS was used to apply Univariate Cox Proportional Hazards Models, investigating how the variables gender, pre-TX status, age, BMI, lymphocytes/monocytes ratio, neutrophil/lymphocytes ratio, ANC, hemoglobin, thrombocytes, LDH, creatinine, bilirubin, protein, albumin, CRP, ANC-regeneration and T-regeneration affected the overall survival and the progression free survival. To compare the different categories of a variable Log-Rank Tests were applied.

Kaplan-Meier Estimators were created for the parameters found to be of significance in the Cox Proportional Hazards Models.

3 RESULTS

3.1 Basic characteristics of the whole study population

For this study, the data of a total of 396 patients were collected. The study population was composed of 158 (30.9%) women and 238 (60.10%) men. (Figure 5)

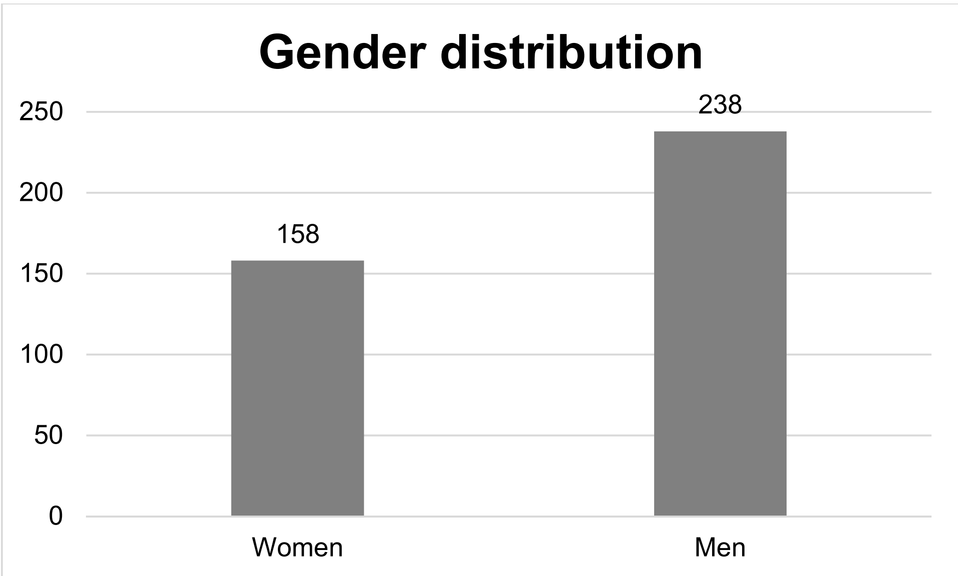

Figure 5: Gender distribution of the general study population consisting of 396 patients.

The study population was roughly divided into two big subcategories of lymphoma – various lymphoma (VL) and Multiple Myeloma (MM). From the total of 396 patients, 164 (41.41%) presented with VL while 232 (58.59%) presented with MM. (Figure 6)

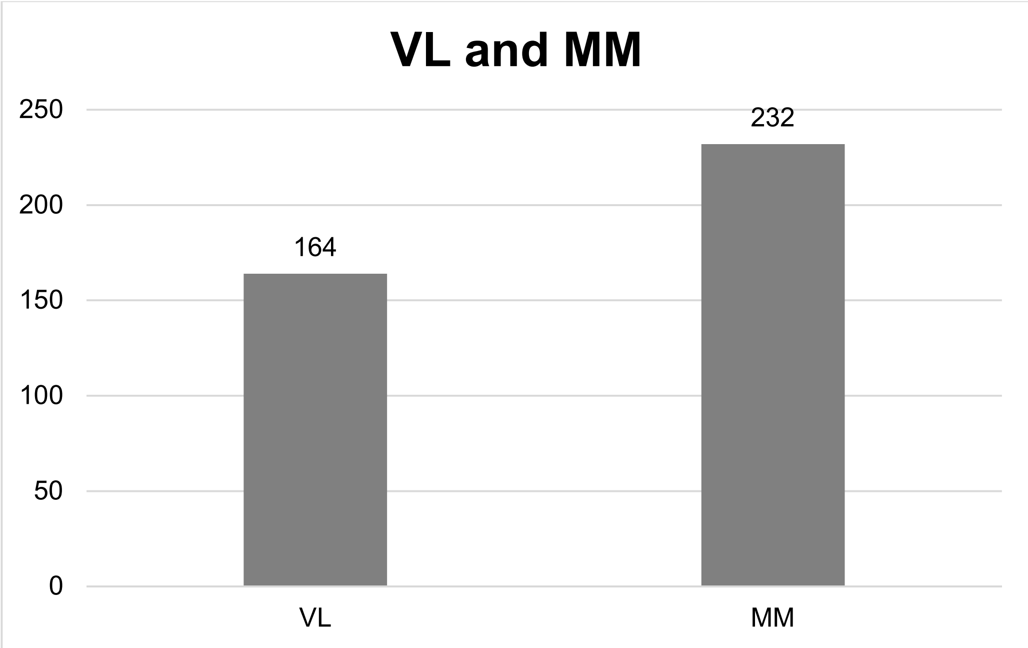

Figure 6: Lymphoma distribution in the study population - division into VL and MM

The large subgroup of VL in this study combines patients suffering from Diffuse large B-cell lymphoma (DLBCL), Mantle cell lymphoma (MCL), Follicular lymphoma (FL), T-cell lymphoma (T-NHL), Chronic lymphocytic leukemia (CLL), Morbus Hodgkin (MH), CNS lymphoma (CNS), Richter's syndrome, Plasma cell leukemia (PCL), Grey zone lymphoma, Burkitt lymphoma and Burkitt-like lymphoma. (Figure 7)

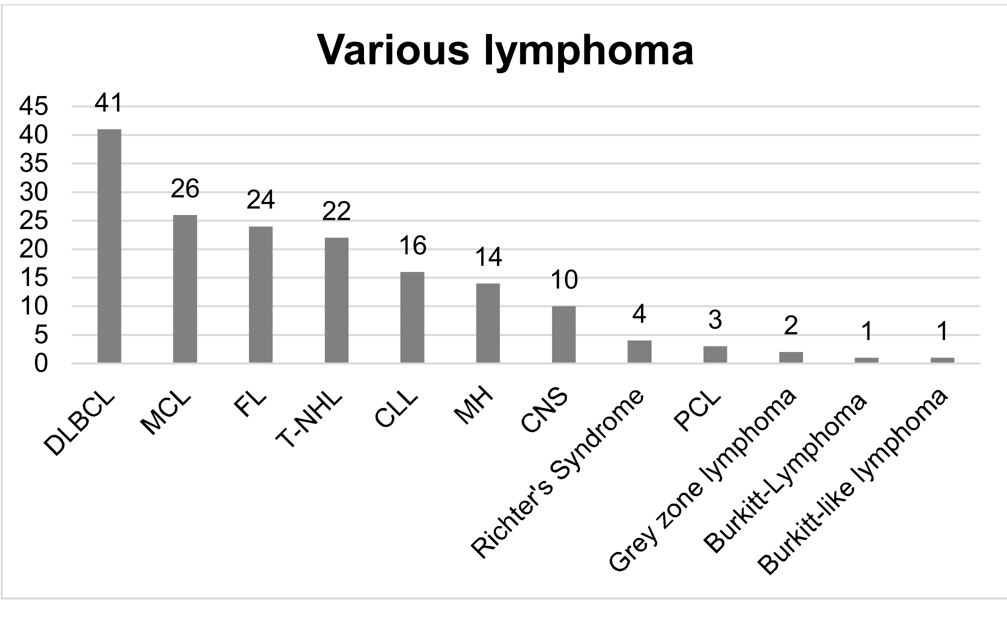

Figure 7: Subcategories making up the big group of various lymphomas in this study

Due to the size of the study population and the diversity of the different diseases the further evaluation concentrates only on the 232 patients suffering from MM.

3.2. Basic characteristics of the MM population

3.2.1 Gender distribution

Out of the total population of 232 patients, 45.3% were female and 54.7% were male. In numbers, the data of 105 women and 127 men, who have been diagnosed with MM, were collected. (Figure 8)

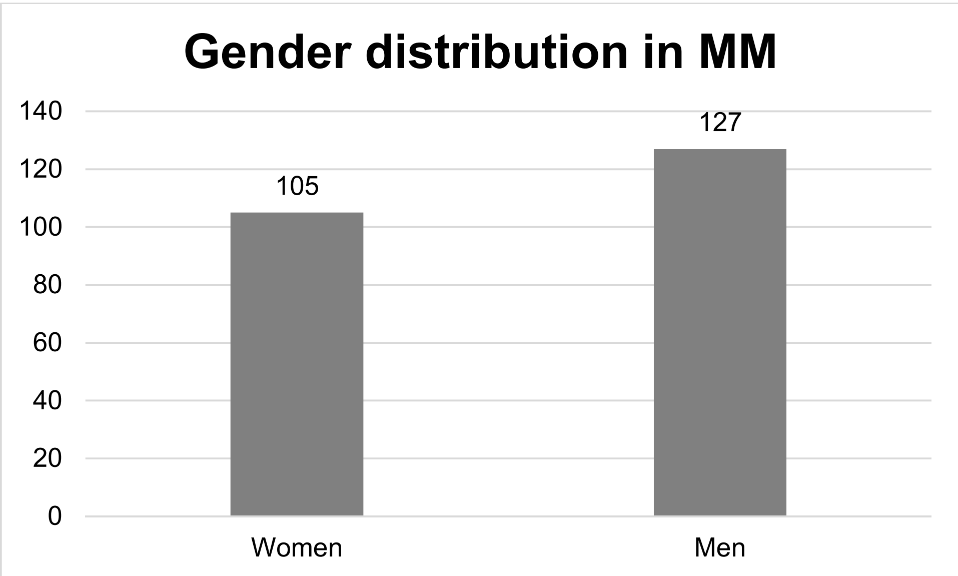

Figure 8: Distribution of genders of the in the study included Myeloma patients

3.2.2 Age distribution

The mean age of patients at TX was 57 years. The youngest patient got transplanted at the age of 30 years. The oldest transplanted patient was 74 years old. (Figure 9)

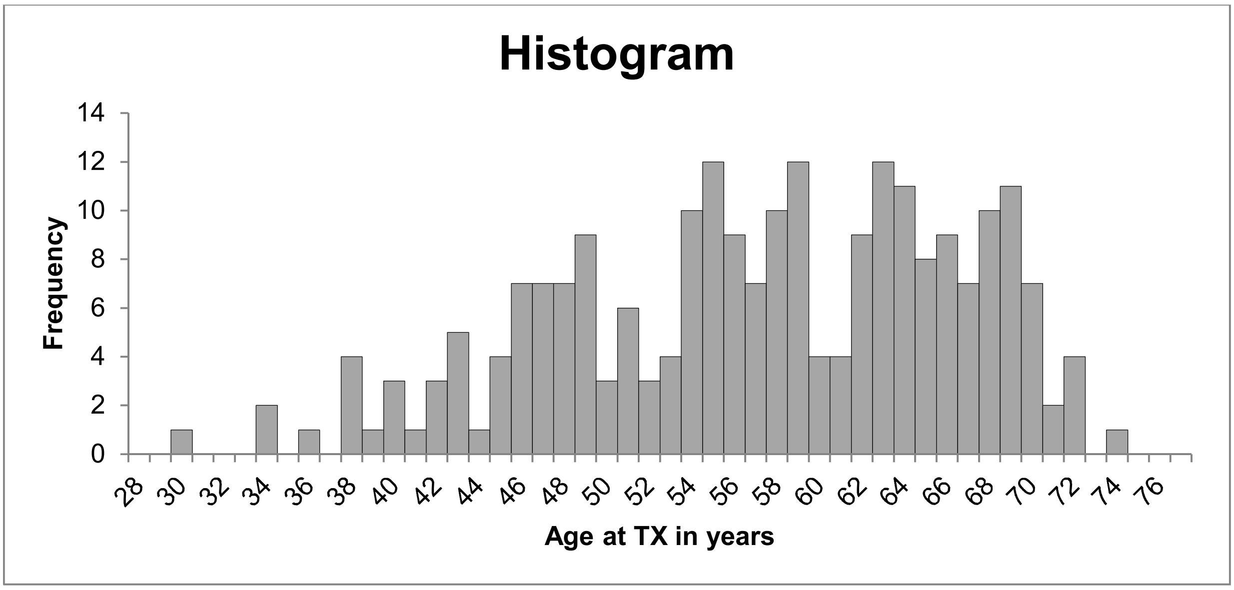

Figure 9: Histogram showing the age distribution of the transplanted patients with a mean age at transplantation of 57 years.

3.2.3 Body Mass Index

The Body Mass Index (BMI) was collected from 202 patients, representing 87.1 % of the total MM study population. In 30 cases, the BMI was not taken down at the patient's admission to the hospital and therefore could not be analysed in this study.

The mean BMI of the total of 202 patients was with 25.98 on the border to pre-obesity (WHO 25.0-29.9). (41)

The lowest BMI registered in a MM patient was 16.0, which is - according to the WHO - considered underweight (<18.5). (41)

The highest BMI could be found at 46.6. A BMI this high indicates obesity class III (WHO >40) (Table 8). (41)

BMI	Nutritional status	Number of patients
< 18.5	Underweight	5
18.5 – 24.9	Normal weight	86
25.0 – 29.9	Pre-obesity	79
30.0 – 34.9	Obesity class I	26
35.0 – 39.9	Obesity Class II	4
> 40	Obesity Class III	2

Table 8: WHO definition of nutritional status depending on the Body Mass Index (BMI) **(41)**

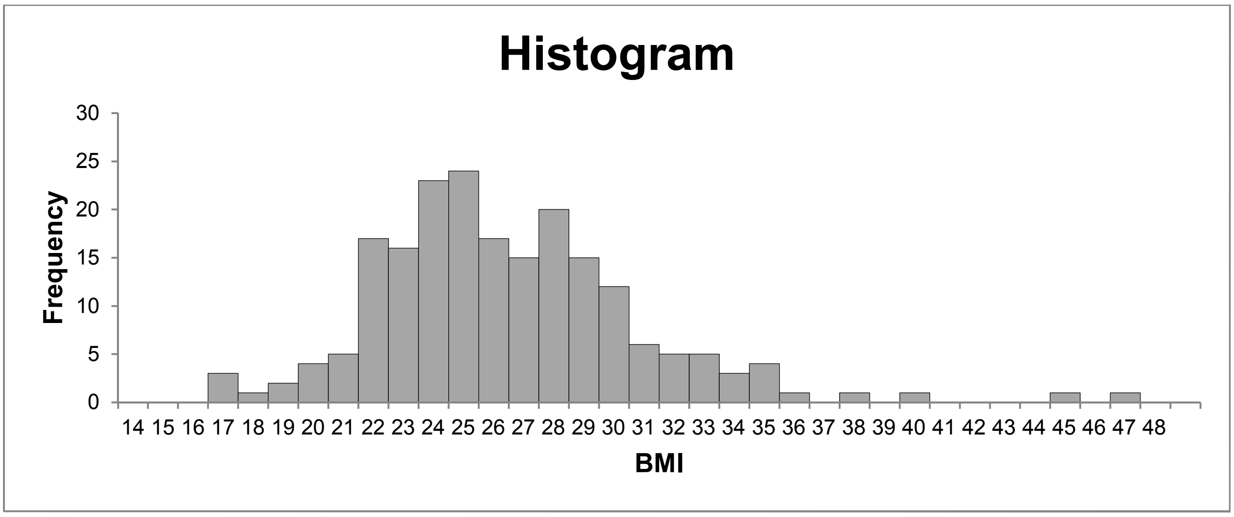

Figure 10: Histogram of the patients' BMI. Values ranged from 17 to 47 resulting in a mean BMI of 25.98.

3.2.4 Laboratory parameters at the day of admission to hospital for TX

For all retrieved laboratory parameters the minimum, maximum, mean, median and standard deviation were calculated. All stated reference values are those used at LKH Graz. A visualisation of the calculated results can be found in Table 9.

Leukocytes

Leukocyte values could be collected for all 232 patients. The reference value for leukocytes lies between 4.4 – 11.3 G/l. 75 patients (32 %) had values lower than the reference value, while 4 (2%) had higher values.

Absolute neutrophil count

The ANC was collected for 231 patients. In one case the count could not be detected. The standard ANC lies between 1.8-7.7 G/l. 16 patients (7%) had levels lower and 9 patients (4%) had levels higher than this reference value.

Lymphocytes

Lymphocyte levels were analysed in 231 of 232 cases. Normal lymphocyte values should lie within a range of 1.0-4.8 G/l. 104 times (45%) a value lower than 1.0 G/l was detected, at the same time no patient had a level higher than the reference spectrum.

Monocytes

Moreover, monocyte levels were collected for 231 out of 232 patients. In one case it was not possible to detect a value. Monocyte levels between 0.2-1.0 G/l are considered normal. 8 values (3%) lay below this range, while 3 (1%) lay above it.

Haemoglobin

Haemoglobin values could be collected for all 232 MM patients of the study population. In 35.3% (a total of 82 patients) levels lower than the reference span of 12.0-17.5 g/dl were taken down.

Thrombocytes

232 sets of thrombocyte values were collected. Thrombocyte values between 140-440 G/l are considered normal. 31 patients (13%) stood out with levels lower than this norm, while only 2 (1%) had values that were too high.

Lactate dehydrogenase

Valid LDH values were found in 230 patient records. In only 2 records this information was missing. Overall only 3 times (1%) levels lower than 120 U/l were taken down, while a total of 35 values (15%) were too high (>240 U/l).

Creatinine

Conclusive creatinine levels could be found in 231 patients. To be considered normal, creatinine values should be at 1.2 mg/dl or lower. 32 patients (14%) showed values higher than this limit.

Bilirubin

Valid bilirubin values could be extracted from 229 patient records. In 3 cases no information could be found. The reference value for bilirubin lies between 0.1-1.2 mg/dl. While no patient had levels lower than that, 7 patients (3%) had values higher than 1.2 mg/dl.

Protein

Suitable protein levels could be retrieved in 225 cases, 97% of the total MM study population. 7 times this data were not collected on the day of admission to the hospital. With normal levels situated between 6.6-8.3 g/dl, 77 values were located outside this range. In 38 cases (16%), detected values were abnormally low, whereas 39 patients (17%) had levels above the upper limit.

Albumin

Albumin values could be gathered from 194 patient records, accounting for 84% of the total MM study population. 38 times the data concerning this parameter were incomplete. Overall only few patients had levels that were abnormally high or low. In only 6 cases (3%) values lay below the lower reference limit, and even less – only 1 patient (0.5%) – exceeded the upper limit.

C-reactive Protein

Valid levels of CRP were extracted from 229 patient records. For 3 patients this information could not be collected. CRP levels up to 5 mg/l or lower are considered normal. 46 patients, accounting for 20% of the study population, had values higher than 5 mg/l.

	Reference value	Median	Median	Maximum	Mean	Standard deviation
Leukocytes	*4.4-11.3 G/l*	0.5	5.0	39.4	5.49	2.97
ANC	*1.8-7.7 G/l*	0.0	3.2	34.6	3.72	2.64
Lymphocytes	*1.0-4.8 G/l*	0.0	1.0	4.0	1.11	0.55
Monocytes	*0.2-1.0 G/l*	0.0	0.4	1.2	0.47	0.20
Haemoglobin	*12.0-17.5 g/dl*	8.2	12.6	16.9	12.52	1.59
Thrombocytes	*140-440 G/l*	6.0	215.0	476.0	217.86	75.39
LDH	*120-240 U/l*	103.0	191.5	1,311.0	210.43	107.99
Creatinine	*≤1.2 mg/dl*	0.5	0.9	5.6	1.02	0.58
Bilirubin	*0.1-1.2 mg/dl*	0.1	0.4	2.2	0.50	0.29
Protein	*6.6-8.3 g/dl*	5.1	7.5	13.1	7.55	1.15
Albumin	*3.5-5.3 g/dl*	2.7	4.3	5.5	4.30	0.44
CRP	*≤5 mg/l*	0.0	1.8	136.8	4.67	12.21

Table 9: Laboratory characteristics of patients with Myeloma

3.3 Transplantation

3.3.1 Type of TX

A total of 217 patients, 93.5% of the total MM study population, underwent autologous stem cell transplantation (ASCT). The remaining 15 patients, representing 6.5% of the MM study population, received allogeneic stem cell transplantation.

Allogeneic TX can be divided into three different types of procedures, taking into account the origin of the donated cells. Cells can be either donated by siblings (sibl.) or unknown donors (matched unrelated donor=MUD). A third way of cell recruitment is the extraction of cells from umbilical cord blood (UCB).

11 patients, 4.7% of all MM patients, received cells from a sibling, while 3, 1.3%, got transplanted allogeneic cells from MUD. UCB was only used in 1 MM patient, accounting for 0.4% of the total population.

3.3.2 Conditioning therapy

ASCT

For conducting the conditioning therapy, several different schemes are in use. In a total of 173 patients, 79,2%, high-dose melphalan (200mg/m2) was used as a conditioning therapy. In 10 out of these 173 patients melphalan 200 was combined with palifermin.

40 times, in 18,4% of patients, 100, 120, 140 or 150 mg/m2 of melphalan were applied. 3 patients, 1.4% of the ASCT study population, were treated according to the so-called BEAM regimen, consisting of carmustine (BCNU), etoposide, AraC and melphalan. (42) The remaining patient underwent conditioning by a combination of bendamustine, etoposide, cytarabine and melphalan – a scheme called BeEAM. (43)

Allogeneic TX

In 9 cases out of 15, 60%, a combination of 90 mg/m2 of fludarabine and 100 mg/m2 of melphalan was the chosen conditioning treatment. 3 patients patient received different combinations of fludarabine, melphalan and antithymocyte-globulin (ATG). (44) One patient was treated with fludarabine, melphalan and BCNU. The remaining 2 patients received 120 mg/kg of cyclophosphamide combined with 11.2 mg/kg of busulfan.

3.3.3 Pre-TX Status

At the time of TX 103 (44.4%) patients were in a state of partial remission (PR), 49 (21.1%) in CR and 34 (14.7%) in very good partial remission (VGPR). In 25 cases (10.8%) the disease was progressing (PD), while 21 patients (9.1%) were at a stable disease status (SD). (Figure 12)

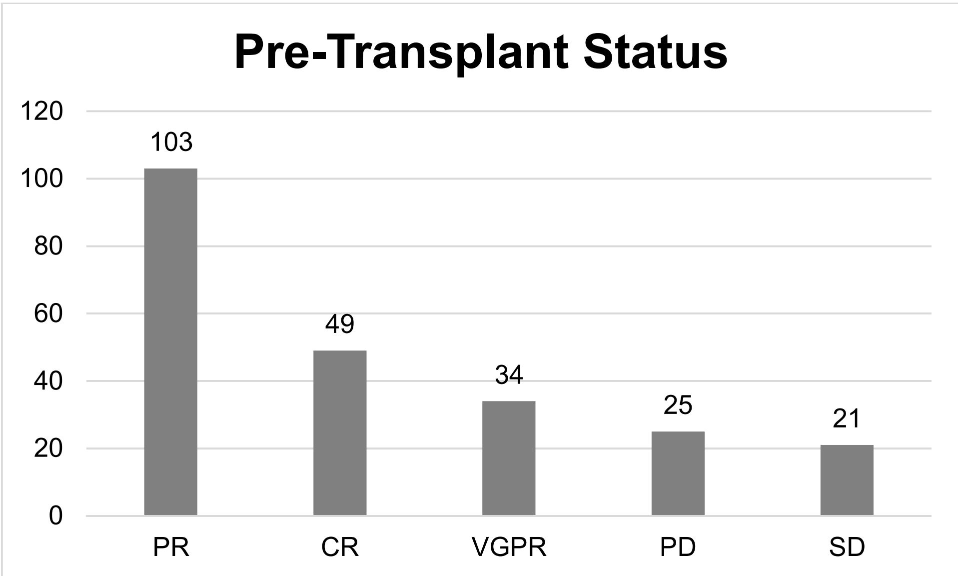

Figure 11: Pre-Transplant Status of Myeloma patients divided into PR, CR, VGPR, PD and SD

3.3.4 Allogeneic TX – additional information

A total of 15 allogeneic stem cell transplantations were performed. Out of these 15 patients, 13 received reduced-intensity conditioning (RIC), while the remaining 2 underwent myeloablative conditioning (MAC).

10 out of 15 donors were female, the rest were male.

The CMV status was collected for 14 out of 15 donors, of which 7 were CMV negative and 7 were CMV positive.

The CMV status of the patient could be detected for all 15 patients. 8 patients reported positive for CMV, while the remaining 7 were CMV negative.

11 times the patient and his / her donor had the same CMV status. Out of these 11 cases 5 times both reported CMV negative and 6 times both were CMV positive. 2 CMV positive patients received stem cells from a CMV negative donor. In one case a CMV negative patient was given stem cells from a donor, who had reported CMV positivity.

14 out of 15 allogeneic transplantations (93.3%) were performed with a complete HLA-match of patient and donor. One transplantation was performed with an HLA match of 10 out of 11.

After the TX, 8 patients (53.3%) developed a Graft-versus-host disease (GvHD) while 7 (46.7%) did not. The GvHD affected predominantly the bowel, closely followed by the skin. Liver, stomach, duodenum, oral mucosa and conjunctiva were also affected in some patients.

3.4 Outcome

3.4.1 Overall Survival (OS)

The status dead or alive was detected for the day of the individual patient's last follow up. On this day 155 MM patients (66.8%) were alive, while 77 patients (33.2%) had passed away. (Figure 13)

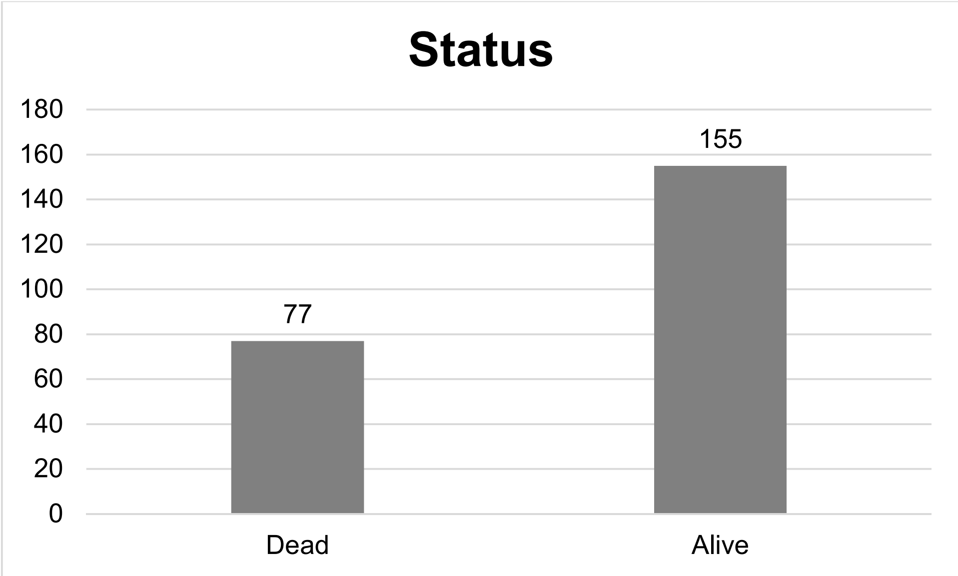

Figure 12: Patients' status at the end of the period of follow up

The shortest period of follow-up lasted for 10 days while the longest amounted up to 4196 days. A median follow-up of 1310.21 days was calculated.

3.4.1.1 Univariate Cox Proportional Hazards Model and Kaplan Meier Estimators

To evaluate the influence of a variable on the OS, a Univariate Cox Proportional Hazards Model was created (Table 10).

In this analysis three parameters, namely the lymphocyte/monocyte-ratio (LMR), the haemoglobin and the thrombocyte value were found to correlate significantly with the OS.

However no evidence for a significant relation between the patient's gender, pre-Tx status, age, BMI, neutrophil/lymphocyte-ratio, ANC, LDH, creatinine, bilirubin, protein, albumin, CRP, ANC-regeneration and T-regeneration and improved OS could be provided.

Variable	Category	Events/N	HR	CI	p-value
Gender	f	38/105	1		0.3235
	m	39/127	0.80	[0.51-1.25]	
Pre-TX-status	CR/PR/VGPR	60/186	1		0.3733
	PD/SD	17/46	1.28	[0.74-2.19]	
Age at TX	[19,65]	62/173	1		0.5749
	[65,74]	15/59	0.85	[0.48-1.50]	
BMI	[16,20]	4/10	1		0.1922
	[20,25]	25/85	0.44	[0.15-1.27]	
	[25,47]	31/107	0.38	[0.13-1.08]	
Lymphocyte/ Monocyte-Ratio	[0,1.2]	11/26	1		**0.0452**
	[1.2,75.6]	66/204	0.52	[0.27-0.99]	
Neutrophil/ Lymphocyte-Ratio	[0.082,2.8]	30/91	1		0.9161
	[2.8,88.7]	47/140	0.98	[0.62-1.54]	
ANC	[0.03,1.5]	1/7	1		0.8037
	[1.5,34.6]	76/224	1.28	[0.18-9.28]	
Haemoglobin	[5.7,12]	34/82	1		**0.0238**
	[12,16.9]	43/150	0.59	[0.38-0.93]	
Thrombocytes	[6,100]	9/13	1		**<0.001**
	[100,549]	68/219	0.12	[0.05-0.24]	
LDH	[83,240]	65/195	1		0.5899
	[0.51-1.25]	12/35	1.18	[0.64-2.20]	
Creatinine	[0.51,1]	48/156	1		0.1303
	[1,5.6]	29/75	1.43	[0.90-2.27]	
Bilirubin	[0.12,1.2]	73/222	1		0.5417
	[1.2,2.8]	3/7	1.44	[0.45-4.60]	
Protein	[5.1,6.6]	15/38	1		0.4222
	[6.6,13.1]	60/187	0.79	[0.45-1.40]	
Albumin	[2.7,3.5]	2/6	1		0.6550
	[3.5,5.5]	61/188	0.72	[0.18-2.97]	
CRP	[0,5]	54/179	1		0.2002
	[5,231]	22/50	1.39	[0.84-2.29]	
ANC-Regeneration	[5,10]	3/6	1		0.2078
	[10,45]	72/224	0.47	[1.51]	
T-Regeneration	[6,10]	13/42	1		0.3853
	[10,59]	59/184	1.30	[0.72-2.38]	

Table 10: Univariate Cox Proportional Hazards Model for OS in MM. LMR, haemoglobin and thrombocytes were found to be of significance and their p-value was therefore marked in bold.

In order to further illustrate the findings, Kaplan-Meier estimators were created for LMR, haemoglobin and thrombocytes.

To analyse the LMR, values lower than 1.2 were separated from values over 1.2. The calculation presented a significantly (p=0.0452) superior OS for patients with a ratio higher than 1.2 on their day of admission, than for those with a lower LMR. (Figure 14)

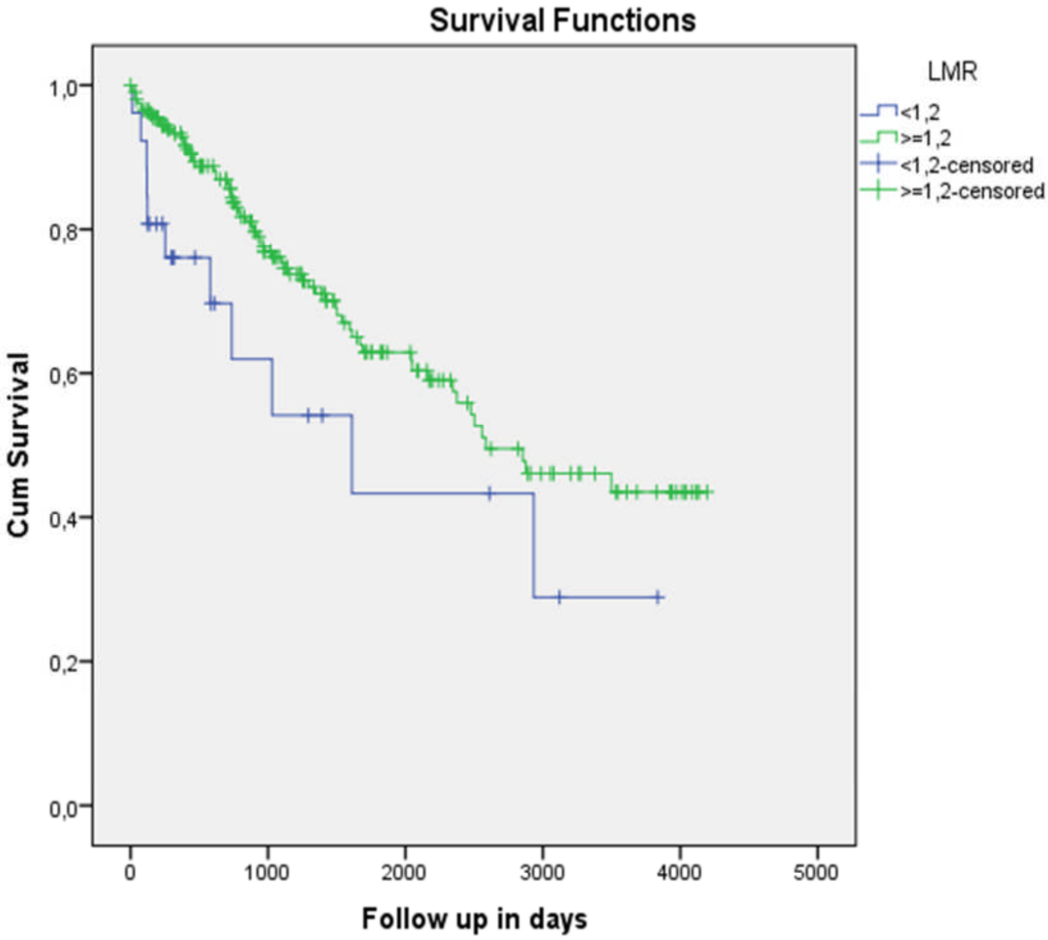

Figure 13: Kaplan-Meier estimator illustrating the influence of low (<1.2) vs. high (≥1.2) LMR on OS. A significantly (p=0.0452) better outcome was found for patients with a pre-transplantational ratio higher than 1.2 in comparison to those with a lower LMR.

In order to evaluate the effect of haemoglobin levels on patients' OS, two different groups were established. Haemoglobin values lower than 12 g/dl were joined to a first group and compared to a second group that included all values higher than 12 g/dl. The analysis revealed that the second group had a significantly (p=0.0238) better OS than the first group. (Figure 15)

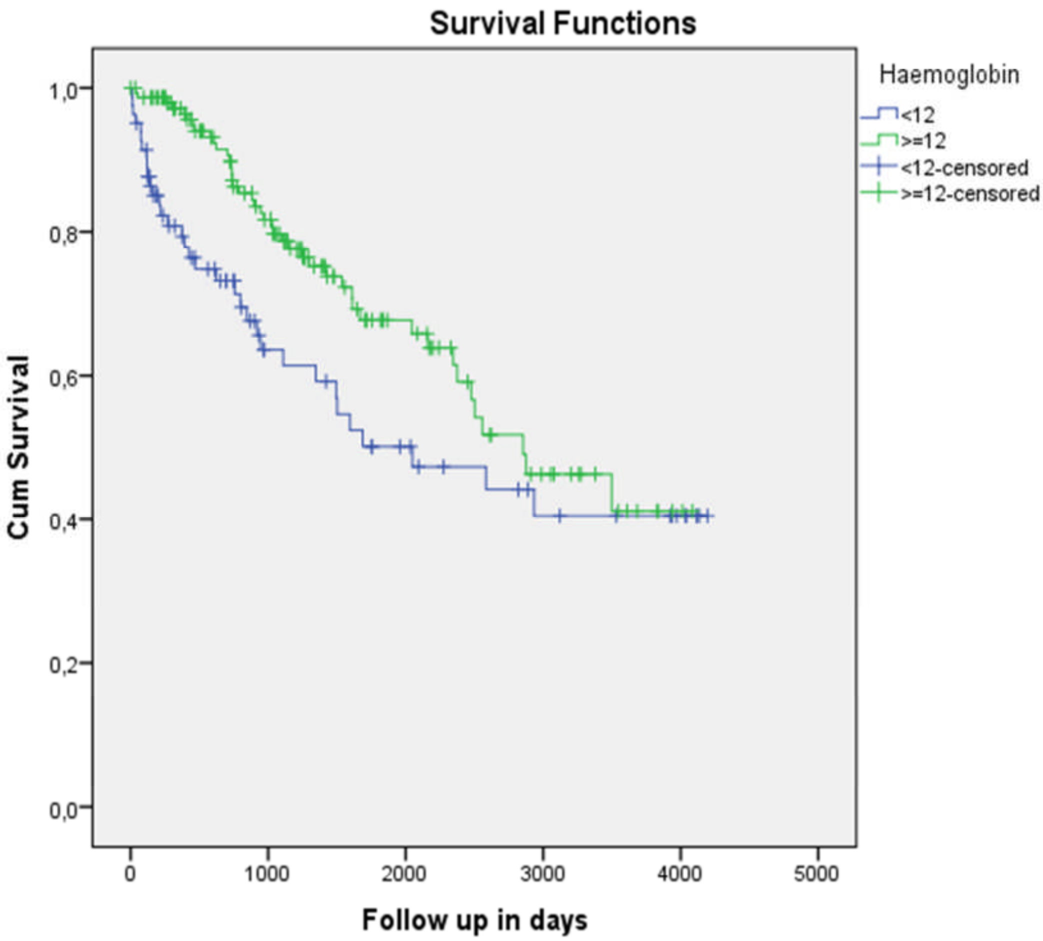

Figure 14: Kaplan-Meier estimator showing the correlation between normal (≥12 g/dl) vs low (<12 g/dl) haemoglobin values and OS. Non anaemic patients with pre-transplantational haemoglobin values higher than 12 g/dl presented with longer OS rates than those with lower levels.

Thrombocyte levels lower than 100 G/l were distinguished and checked against those higher or equal 100 G/l. With a p-value of <0.001 higher values came along with a better outcome concerning the OS. (Figure 16)

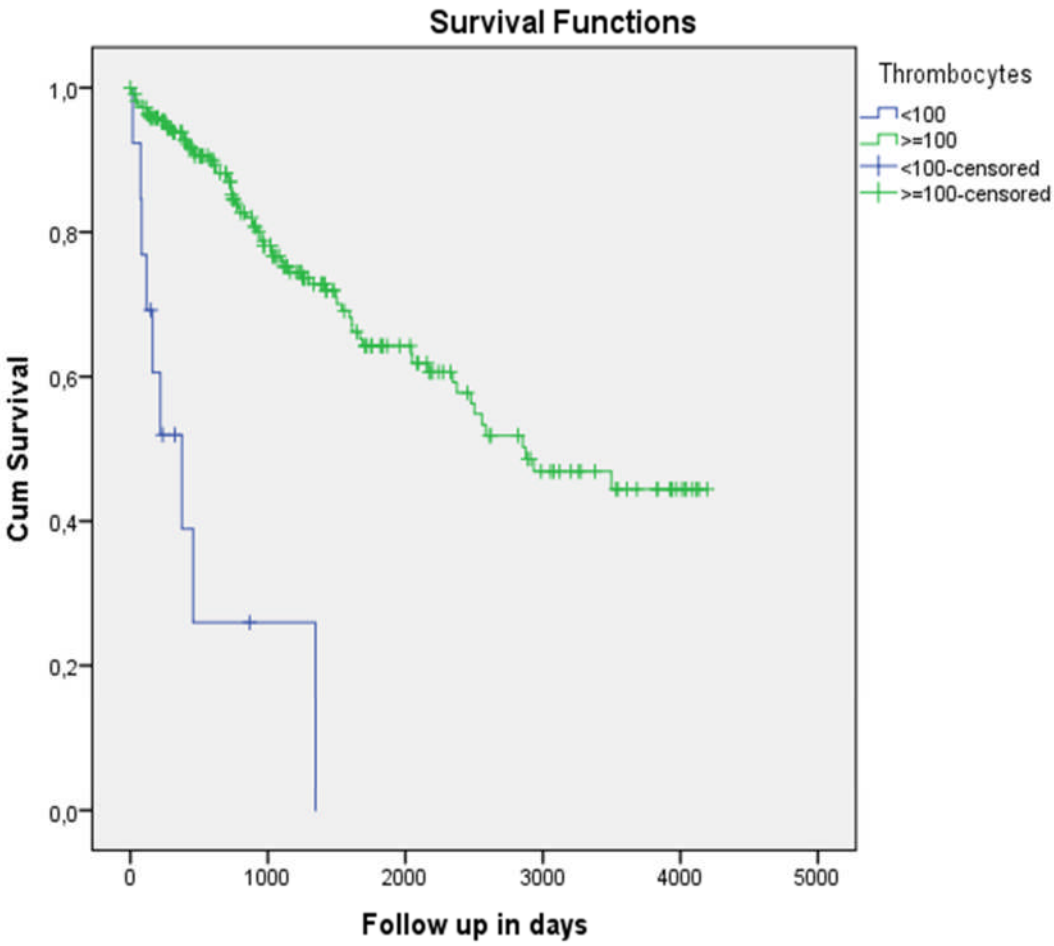

Figure 15: Correlation between normal (≥100 G/l) vs reduced (<100 G/l) thrombocyte values and OS visualised in a Kaplan-Meier estimator. A statistically significant ($p<0.001$) association between normal thrombocyte levels (≥100 G/l) and better OS was found.

3.4.2 Progression-Free Survival (PFS)

129 patients (55.6%) suffered a relapse, whereas the remaining 103 patients (44.4%) did not. 69 out of 127 men relapsed, representing a percentage of 54.3%. In the female population 57.1%, a total of 60 women, developed a relapse. (Figure 17)

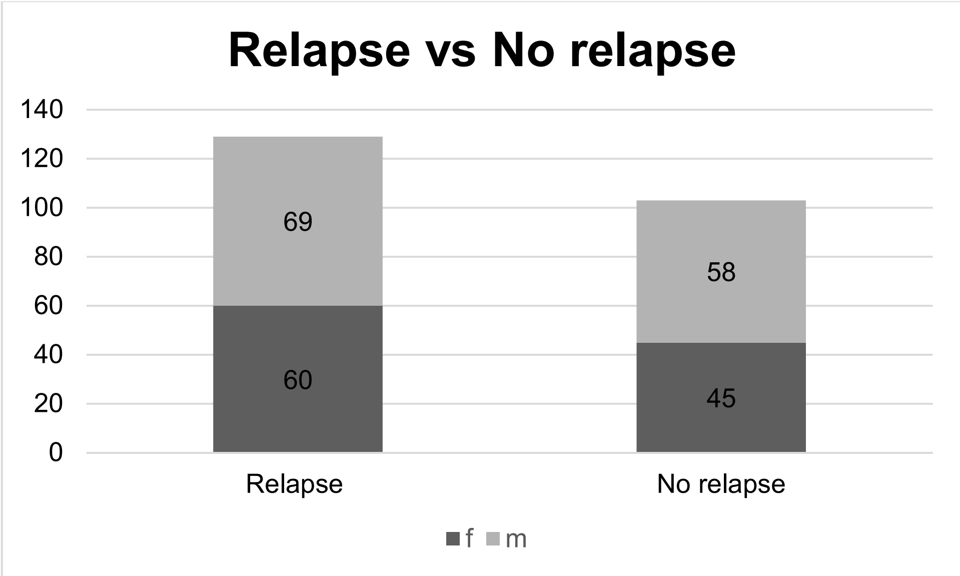

Figure 16: Distribution of patients concerning the occurrence of a relapse

3.4.2.1 Univariate Cox Proportional Hazards Model and Kaplan-Meier estimator

When looking at PFS after TX, the Univariate Cox Proportional Hazards Model demonstrated that only one parameter significantly influenced PFS.

While the evaluation of gender, pre-TX status, age, BMI, LMR, neutrophil/lymphocyte ratio, ANC, haemoglobin, thrombocytes, LDH, creatinine, bilirubin, protein, albumin, CRP, ANC-regeneration and T- regeneration failed to come up with significant results, the analysis of CRP levels provided evidence of this parameter correlating with outcome in terms of PFS. (Table 11)

Variable	Category	Events/N	HR	CI	p-value
Gender	f	60/105	1		0.2854
	m	69/127	0.83	[0.58-1.17]	
Pre-TX-status	CR/PR/VGPR	104/186	1		0.9711
	PD/SD	25/46	1.01	[0.65-1.57]	
Age at TX	[19,65]	97/173	1		0.4936
	[65,74]	32/59	1.15	[0.77-1.72]	
BMI	[16,20]	6/10	1		0.2510
	[20,25]	48/85	0.63	[0.27-1.49]	
	[25,47]	59/107	0.52	[0.22-1.22]	
Lymphocyte/ Monocyte-Ratio	[0,1.2]	14/26	1		0.1979
	[1.2,75.6]	114/204	0.69	[0.40-1.21]	
Neutrophil/ Lymphocyte-Ratio	[0.082,2.8]	49/91	1		0.4272
	[2.8,88.7]	80/140	1.16	[0.81-1.65]	
ANC	[0.03,1.5]	3/7	1		0.9872
	[1.5,34.6]	126/224	1.01	[0.32-3.18]	
Haemoglobin	[5.7,12]	45/82	1		0.3158
	[12,16.9]	84/150	0.83	[0.58-1.19]	
Thrombocytes	[6,100]	4/13	1		0.4562
	[100,549]	125/219	0.68	[0.25-1.86]	
LDH	[83,240]	109/195	1		0.7377
	[0.51-1.25]	20/35	1.09	[0.67-1.75]	
Creatinine	[0.51,1]	93/156	1		0.4916
	[1,5.6]	36/75	0.87	[0.59-1.28]	
Bilirubin	[0.12,1.2]	123/222	1		0.7181
	[1.2,2.8]	5/7	1.18	[0.48-2.90]	
Protein	[5.1,6.6]	26/38	1		0.4767
	[6.6,13.1]	100/187	0.85	[0.55-1.32]	
Albumin	[2.7,3.5]	3/6	1		0.2690
	[3.5,5.5]	106/188	0.52	[0.16-1.65]	
CRP	[0,5]	106/179	1		**0.0262**
	[5,231]	22/50	0.59	[0.37-0.94]	
ANC-Regeneration	[5,10]	2/6	1		0.4034
	[10,45]	127/224	1.82	[0.45-7.35]	
T-Regeneration	[6,10]	27/42	1		0.9511
	[10,59]	101/184	0.99	[0.64-1.51]	

Table 11: Univariate Cox Hazards Model for PFS in MM. High CRP was found to be associated with PFS. For better visualisation the respective p-value was marked in bold.

Normal CRP levels (<5 mg/l) were separated from levels higher than that reference limit. With a p-value of 0.0262 CRP levels lower than 5 mg/l were calculated to result in an inferior outcome concerning PFS. (Figure 18)

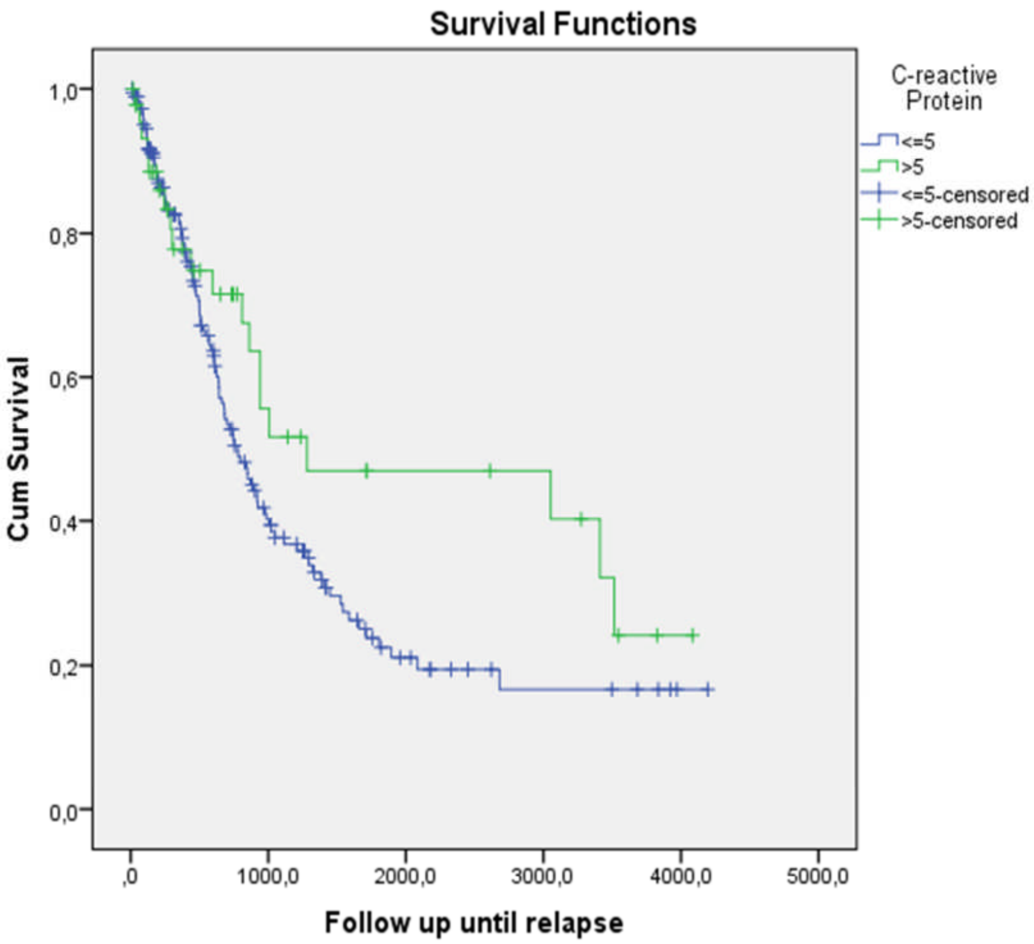

Figure 17: Kaplan-Meier estimator for PFS comparing normal (≤5 mg/l) vs elevated (>5 G/l) C-reactive protein values. Normal CRP levels were shown to come along with significantly (p=0.0262) inferior PFS compared to elevated CRP levels.

4 DISCUSSION

Multiple Myeloma ranks, according to several statistics, among the 2 to 3 most common types of blood cancer. (45,46) With around 114,000 newly diagnosed cases worldwide in 2012, MM was – accounting for 0.8% of the total cancer population - considered the 23rd most prevalent cancer in the world. (47)

Concerning gender distribution, some sources report a slight emphasis on the male gender (3), while others suggest that men and women are equally affected. (5) With around 55% of the study population being male and only 45% being female, the results of this thesis conform with the first proposition.

With an incidence rate increasing after the age of 60, MM is considered a disease of advanced age. (3) Due to the fact that this analysis was aimed at therapy-optimisation it does not come up with data about the age of onset. A mean age at transplantation of 57 years, goes along with the age distribution mentioned above, especially considering the fact that transplantion is mostly used in younger patients.

Throughout the observation period, about 33% of all patients died, while 67% were still alive at the end of our evaluation. About 56% of all patients suffered a relapse, the remaining 44% survived without progression.

Augmented research performed over the last decade, led to the development of new treatment strategies and to further improvement of well-tested concepts of the therapy of MM, resulting in constantly increasing survival rates. (46)
217 of the 232 patients, suffering from MM treated with TX at the LKH Graz and the LKH Hochsteiermark, underwent an autologous approach. Only 15 patients (6.5%) were treated with allogeneic stem cell transplantation. Although the allogeneic approach can potentially entirely cure a patient from the disease it is not used as frequently as the autologous alternative. (12) One of the reasons for that is the fact that allogeneic stem cell transplantation carries the risk of a large number of dangerous side effects and therefore can only be administered to very few, highly selected patients. (12)

GvHD being one of these side effects often complicates the healing process and further life of transplanted patients. In this study population GvHD aggravated the further course of as many as 50% of patients.
For a long time, TX has been the only method of therapy that could possibly reach a VGPR or CR in a quite big amount of patients. (48) The great advances of the last years that established new potent substances like proteasome inhibitors for induction therapy managed to reach VGPR even before TX in up to 60% of patients. These new findings have resulted in the need of a reevaluation of the role of TX in MM patients. (48) Due to the fact that the procedure still comes with high risks for the patient, prognostic indicators should be analysed more detailed.
The aim of this thesis was to identify parameters predicting and influencing PFS and OS rates of transplanted patients. Such parameters should possibly be measured and monitored easily and fast and, even more important, modified easily.
By detecting such indicators, risks of the TX could be minimized and the outcome of MM patients after TX could be improved.
For this purpose a large number of simple laboratory parameters as well as other factors including gender, age at transplantation, body mass index and pre-transplantation status were analyzed in this thesis.
In this study, gender and age at TX were found not to correlate statistically with OS nor with PFS. This is most likely due to the large diversity and complexity of the other parameters that possibly influence the outcome stronger than gender and age at TX alone ever could.

Obesity defined by a high BMI is considered a risk factor as well as a prognostic parameter for inferior outcome in a lot of different medical situations. Hence it was rather surprising that a high BMI was found not to be associated with a worse PFS or OS. This finding might base upon the fact that recovering from transplantation is a long and draining process were a patient might benefit from having some physical and nutritional reserves.

With a p-value of 0.3733 for OS and 0.9711 for PFS the pre-TX-status failed to be of statistical significance as well. This contrasts several other studies that report the opposite in myeloma patients.(49,50) Lahuerte JJ. et al., for instance, analysed the influence of pre- and post-TX response on the outcome – event-free survival (EFS) and OS - of 632 myeloma patients. They reported a strong correlation of pre- and post-TX CR and longer EFS and OS. Furthermore they pointed out to which large extent pre-TX response influences post-TX response. (49) Another study, conducted by O'Shea D. et al., with a similar aim as this thesis, also reports a strong association between pre-TX CR and longer EFS. (50) An explanation for the fact that this thesis could not come up with a similar conclusion may be found in the heterogenity of our study population.

The analysed laboratory parameters provided more promising results. Decreased levels of haemoglobin and thrombocytes as well as a low ratio of lymphocytes and monocytes were found to be associated with inferior OS.

Haemoglobin changes and especially low haemoglobin levels and anaemia have already been identified as a prognostic variable in many types of cancer including lung cancer, breast cancer, colorectal carcinoma and several more. (51,52)
This proposition also applies to haematologic malignancies. An extensive literature review by Caro J. et al. in 2001, for instance, discovered a relative increase as high as 67% in death risk in patients with anaemia with lymphoma compared to patients with lymphoma without anaemia. (52)
Anaemia is a common finding in patients with cancer and an association with poorer outcome might not be surprising. In this study we not only looked at low haemoglobin in general but at low haemoglobin at the time of TX. A significantly better OS (p=0.0238) for patients with haemoglobin values higher than 12 g/dl compared to those with values lower than that was detected. These findings could suggest that - in an optimal course - patients may benefit from undergoing TX at a time where their haemoglobin levels are as close to normal as possible. However this approach is most of the times only limited realisable in an acute clinical setting.

Several papers discuss the significance of thrombocytosis in the OS in different malignancies. With platelets contributing actively to carcinogenesis and metastatic spread, thrombocytosis (53) is considered a prognostic marker for poor outcome in a variety of malignancies including breast cancer (54), ovarian carcinoma (55) and gastrointestinal cancers (53).

When talking about TX the opposite is the case. Sustained thrombocytopenia is considered a complication of TX and a well known risk factor for poor prognosis. Therefore it might not be surprising that thrombocytopenia in a patient at the time of TX can represent a poor precondition. This thesis now suggests that thrombocyte levels higher or equal 100 G/l are significantly ($p<0.0001$) superior concerning OS than lower values.

In 2014 and 2015 two studies revealed the predictive value of LMR on the outcome after ASCT in diffuse large B-cell lymphoma (56) and classical Hodgkin lymphoma (57). In both diseases, an LMR higher or equal 1.0 was associated with better OS. However in May / June 2016 a small study was published analysing the predictive potential of the ratio in MM patients with ASCT. In that study no correlation between higher / lower LMR and superior / inferior OS could be found. (58) This thesis now suggests that with a p-value of 0.0452 an LMR greater than 1.2 is significantly associated with a longer OS.

A recent study discusses the prognostic significance of LMR in myeloma patients at the time of diagnosis. Shin SJ et al. report that a low LMR is associated with shorter OS in their study group of 189 patients. Only about a third of their patients were treated with TX, therefore they did not manage to make a clear statement on LMR in transplantation patients. (59) The significance of the absolute lymphocyte count (ALC) separately has also been adressed in several studies. The ALC was found to be a prognostic indicator for survival in newly diagnosed myeloma patients. (60) In addition, an early, proper recovery of the ALC was found to be a predictor for superior OS and PFS after ASCT in MM patients. (61) This thesis, however, could not reach any results that support those findings on ALC.

When looking at the PFS, only CRP turned out to be of significance.

CRP, which can be measured easily, fast and cheap, is the perfect example for a parameter fulfilling the requirements of this study. In Europe it is frequently collected to discover infections in most routine blood examinations and can even be measured from capillary blood, making it accessible even in acute situations in severely sick patients.

Many studies provide evidence of a direct link between high baseline CRP levels and general mortality. (62)
In oncology, high CRP levels were found to be associated with a higher incidence of cancer in cancer-free patients (63) and poorer outcome in many cancers, including diffuse large B-cell lymphoma (64), small cell lung cancer (65) and colon cancer (66) amongst many others.
Although all these findings indicate a similar significance of CRP in MM patients, this supposition has not yet been verified in a large central European study. This thesis now even went a step further and tried to come up with results concerning the significance of CRP in the setting of MM patients at the time of TX.
What many studies already proved for a large number of other malignancies (64–66), the statistical analysis in this case, however, failed to provide. No evidence of a direct association between high CRP levels before TX and inferior OS could be found.
When looking at PFS, however, with a p-value of 0.0262 a statistically significant correlation between CRP values higher than 5mg/l and longer PFS was detected.
This proven prognostic relevance of elevated CRP, contrasts the results of an article on CRP in DLBCL by Troppan et al. mentioned above and the data of many other studies on haematologic malignancies.(64,67) In DLBCL, for instance, high CRP levels at the time of diagnosis were identified as a poor prognostic indicator concerning OS as well as DFS (disease-free survival). (64)
A possible explanation for this role of the CRP can be found in a paper by Yang et al.. They demonstrated that CRP augments the stress- associated growth and spread of myeloma cells. Further they proved that CRP protects myeloma cells from chemotherapy drug-induced apoptosis through several different pathways. (64,67)
Why this study now came up with such conflictive results, still has to be analysed in thorough investigations. A search for related literatur, resulted in the discovery of at least one more abstract with similar findings. In a recently published abstract in *Blood*, Gunji et al. reported an association of elevated CRP levels with longer survival in peripheral T-cell lymphoma, not otherwise specified. (68)

On grounds of the results mentioned above, the primary aim of this thesis, to find easily alterable prognostic indicators for the outcome of MM patients undergoing TX, was achieved.

Since the cohort of this thesis only comprised 232 myeloma patients, confirmation in a study with a larger group of patients might be of value, especially in a prospective setting. Following steps could also include coming up with appropriate ways to use the influence of the parameters and evaluating different approaches to their modification in further studies.

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