AUTOLOGOUS STEM CELL TRANSPLANTATION IN ADULT MULTIPLE MYELOMA PATIENTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine)

30th September 2024

Johannesburg

ETHICS COMMITTEE APPROVAL

This study was approved by the Human Research Ethics Committee (HREC) (Medical), of the University of the Witwatersrand. Clearance certificate number: M210739. Approval was later granted by the HREC (Protocol reference number: M210739) to extend the study inclusion period by 12 months (See appendices A and B)

DECLARATION

I declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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(Signature of candidate)

On the 30th of September 2024, at Johannesburg.

DEDICATION

This research report is sincerely dedicated to my lovely wife Loona and my daughter Poshika, who believed in me and supported me every step of the way.

ACKOWLEDGEMENTS

I would like to convey my gratitude to my supervisors, Professor M. Patel and Dr A. Lakha for their inestimable support and guidance throughout this research project.

I would also like to express my deepest appreciation to the following people for their contribution in bringing this study to fruition:

Mrs Delene Stevens, the Clinical Haematology Unit secretary, for compiling the list of patients used in the study and for allowing me access to the records room over weekends.

The nurses and clerks at the Haematology clinic for helping with acquisition of patient files.

The statistician for doing an impressive job at compiling and analysing all the data used for the study.

ABSTRACT

a. Background:

Multiple myeloma (MM) is a haematological malignancy that results from the unchecked proliferation of a clone of antibody-producing plasma cells. The disease is characterised by the appearance of the myeloma protein (M protein), which is seen in up to 97% of MM patients, and the clinical features are a consequence of end-organ involvement, especially bone and kidneys, by the M protein. Males are afflicted by the disease slightly more commonly, with a male: female ratio of 1.6:1 and black individuals have a two-fold increased prevalence compared to their white counterparts. The median age of patients at diagnosis is 65 years in the USA and 72 years in Europe. A local study done in sub-Saharan Africa showed that the mean age at diagnosis was 61.4 years and that 7.1% of newly diagnosed MM patients were less than 40 years of age. At Chris Hani Baragwanath Academic Hospital (CHBAH), the survival of newly diagnosed MM patients was shown to be < 50% within the first year after diagnosis, highlighting the dismal prognosis of this disease in our setting.

For the past 40 years, high-dose chemotherapy with melphalan followed by autologous stem cell transplantation (ASCT) has been the standard of care globally for eligible patients < 65 years old and improves survival outcomes significantly. At CHBAH, high-dose chemotherapy with ASCT for MM patients has been used since 2003. This consolidation therapy is preceded by induction chemotherapy with a combination of different drugs to decrease the tumour burden and achieve a satisfactory response of the disease. The introduction of novel agents such as proteasome inhibitors (PI) and immunomodulatory drugs (IMiD) about 25 years ago has not superseded but, instead, further strengthened the role of ASCT as a central treatment modality (consolidation) in MM patients. Early upfront ASCT is associated with better survival outcomes than a delayed approach and is the standard of care in most centres across the world.

This study was undertaken to analyse the demographic, clinical, laboratory and radiological findings as well as the complications, treatment outcome and survival of adult MM patients who underwent an ASCT at our treatment facility.

b. Patients and methods

This was a retrospective study of all adult patients with a confirmed diagnosis of MM who had an ASCT at the Clinical Haematology Unit, Department of Medicine, CHBAH over a 19-year period (01/01/2003 to 31/12/2021). Demographic, clinical, radiological and therapeutic data was retrieved from the patient files and laboratory data from the NHLS database. Data was captured on a spreadsheet using Microsoft Excel and was then statistically analysed using SAS Enterprise Guide 7.1. Categorical data was analysed and presented as frequencies and percentages for the overall study. Continuous measures were assessed using medians and inter-quartile ranges (IQR) as well as means and standard deviations and compared non-parametrically and parametrically using the Kruskal-Wallis test and student t-test, respectively.

c. Results and discussion

During the study period, a total of 691 patients were diagnosed with MM. 51 (7.4%) of these patients received an ASCT at our facility. There were three patients who received an ASCT at another centre and were therefore not included in the study. 30 (58.8%) females and 21 (41.2%) males received an ASCT. The male:female ratio was 1:1.43. The median age at presentation was 54 years (range 32-62 years). Black patients (86.3%) represented the major ethnicity to receive an ASCT. 76.5% of the transplanted patients had an ECOG performance status less than two.

The key findings in our review were as follows:

1. The mean number of patients who received an ASCT per year was 2.68, with a median of three. The highest number of ASCT in a year was seven in the year 2019. The mean number of transplants in the second half of the study period (July 2012 to December 2021) was higher at 3.3 compared to the first half of the study, which was two.
2. The most common co-morbidity was hypertension (64.7%), followed by diabetes (19.6%). 29.4% had more than one co-morbidity. 7.8% had some degree of renal impairment at diagnosis (none required dialysis). Three patients had concomitant HIV infection, which was virologically supressed before their ASCT.
3. The most common presenting symptom was bone pain (86.3%); Anaemia (haemoglobin concentration of ≤ 12 g/dl) was seen in 83.3%.
4. The baseline median haemoglobin level was 9.65 g/dl, albumin 35 g/l, creatinine. 82 mmol/l, calcium 2.4 mmol/l, beta 2 microglobulin 4.9 mg/l and LDH 256 U/l.
5. The following improvements in mean laboratory indices were seen one year after ASCT:
    i. Haemoglobin: 9.65 g/dl to 12.3 g/dl.
    ii. Total serum protein: 93 g/l to 77 g/l.
    iii. Serum albumin: 35 g/l to 42 g/l.
    iv. Serum paraprotein level: 38 g/l to 9 g/l.
6. Thirty-two % of the patients were classified ISS I, 32 % ISS II and 36 % ISS III; 94 % had Durie-Salmon stage III disease.
7. CVAD was the most commonly used induction regimen (85.1%); a median of eight induction chemotherapy cycles was given before ASCT; the median time between completing induction chemotherapy and receiving ASCT was six months, with a mean of 10.5 months for the entire cohort.
8. Etoposide was used as mobilisation agent in all of our patients; the median $CD34^+$ yield obtained for ASCT was 9.83 x 10^6/kg (range 2-36 x 10^6/kg).

9. Melphalan 200 mg/m^2 was used in 80% of the patients as conditioning regimen prior to ASCT.
10. Neutrophil engraftment occurred after a median of 11 days; platelet engraftment occurred after a median of 10 days.
11. Forty-one patients received a single ASCT; 10 patients received a second ASCT as salvage therapy following disease relapse. The majority of the patients (74.5%) received a delayed ASCT (>12 months from the date of diagnosis).
12. The most common complication following HDT was neutropenia. Three patients died as a result of transplant-related complications.
13. Thalidomide was the main maintenance chemotherapy agent in our patients. Most patients benefited from thalidomide in the second half of the study (26 versus 3).
14. The median OS was 57 months from the time of diagnosis (range 12-209 months). The median OS in the early ASCT group was 32 months compared to 58.5 months for the delayed ASCT group. In terms of disease response status, 54.5% and 55.5% were in PR or better status in the early and delayed ASCT groups, respectively.
15. Twenty-two patients (43.1%) were alive at the conclusion of the study, out of which 17 (77.2%) were in PR or better disease response status. 56.9% had demised or presumed to be dead as per their last visit notes.
16. Twenty-three patients relapsed at some point after ASCT. The median time for relapse after the first ASCT was 30 months.

d. Conclusions and future recommendations:

Autologous stem cell transplantation remains the standard of care for patients with newly diagnosed MM. Despite the advent of novel agents, ASCT remains a very common treatment modality as consolidation that has consistently shown to improve survival outcomes. It is clear from this study that ASCT improves the outcome of our patient population and its continued use is relevant given the fact that we are still using

outdated induction agents and that novel agents are not widely accessible in the South African public sector. In the South African public sector, only a limited number of health facilities offer ASCT to MM patients.

A recommendation at our treatment facility would be to consider offering ASCT to patients > 65 years of age with a good performance status and minimal co-morbidities as these patients represent a significant proportion of newly diagnosed MM patients, keeping in mind that the life expectancy of the South African population is increasing. Earlier diagnosis and referral to our treatment facility will ensure that these patients stand a better chance of receiving earlier therapy, including ASCT. With regards to this, continued education and training of medical personnel, especially at peripheral hospitals, is of paramount importance.

ASCT has been consistently shown to be of more benefit to MM patients with high-risk cytogenetics. Hence, FISH analysis and cytogenetics on BMAT samples should be offered more widely by the NHLS laboratories across the country. This will require recruitment and training of more personnel to help with this service.

Finally, a concerted effort is needed by the Department of Health and the South African private health sector to make the novel induction agents more accessible to patients at public health sector facilities, as these agents will change the landscape of MM care and significantly improve outcomes by improving the quality of the initial induction response, by allowing us a wider selection of maintenance therapy agents, by allowing a greater access to therapies in the relapsed setting and ultimately with the goal of improving overall survival in patients with MM.

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AKI	Acute kidney injury
ASCT	Autologous stem cell transplantation
BCL-2	B-cell lymphoma-2
BMAT	Bone marrow aspirate and trephine
BMPC	Bone marrow plasma cells
BMSC	Bone marrow stromal cells
BMT-CTN	Blood and Marrow Transplant Clinical Trials Network
cART	Combination antiretroviral treatment
CD	Cluster of differentiation
CHBAH	Chris Hani Baragwanath Academic Hospital
CR	Complete response
CRAB	hyperCalcaemia, Renal dysfunction, Anaemia and Bone disease
CTD	Cyclophosphamide-Thalidomide-Dexamethasone
CXCR4	Chemokine receptor 4
Dara-VRD	Daratumumab-Bortezomib-Lenalidomide-Dexamethasone
DKK-1	Dickkopf-1
DOAC	Direct oral anticoagulant
DRD	Daratumumab-Lenalidomide-Dexamethasone
DRd	Daratumumab-Lenalidomide-low dose dexamethasone
DVD	Daratumumab-Bortezomib-Dexamethasone
EBMT	European Blood and Marrow Transplantation
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free survival
EMN	European Myeloma Network
FBC	Full blood count
FDA	Food and Drug Administration

FISH	Fluorescent in situ hybridisation
FREQ	Frequency
G-CSF	Granulocyte colony stimulating factor
HDT	High-dose therapy
HIV	Human immunodeficiency virus
HOVON	Stichting Hemato-Oncologie voor Volwassenen Nederland
HR	Hazards ratio
HREC	Human Research Ethics Committee
IFM	Intergroupe Francophone du Myélome
Ig	Immunoglobulin
IgH	Immunoglobulin heavy chain
IL	Interleukin
IMiD	Immunomodulatory drug
IMWG	International myeloma working group
IQR	Interquartile range
ISS	International staging system
LDH	Lactate dehydrogenase
LMWH	Low molecular weight heparin
MDE	Myeloma-defining events
MGUS	Monoclonal gammopathy of undetermined significance
MIP-1α	Macrophage inflammatory protein-1 alpha
MM	Multiple myeloma
MP	Melphalan-Prednisone
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
mSMART	Mayo Stratification of Myeloma and Risk Adapted Therapy
NCCN	National Comprehensive Cancer Network

NHLS	National Health Laboratory Services
NSAID	Nonsteroidal anti-inflammatory drug
OS	Overall survival
PBSC	Peripheral blood stem cells
PET-CT	18F-fluorodeoxyglucose positron emission tomography with computed tomography
PFS	Progression-free survival
PI	Proteasome inhibitor
PR	Partial response
PS	Performance status
RANKL	Receptor activator of nuclear factor kappa B ligand
RD	Lenalidomide-Dexamethasone
Rd	Lenalidomide-low dose dexamethasone
R-ISS	Revised international staging system
SA	South Africa
SDF-1	Stromal-derived factor 1
SFLC	Serum free light chains
SMM	Smouldering multiple myeloma
SPEP	Serum protein electrophoresis
SWOG	Southwest Oncology Group
TNFα	Tumour necrosis factor alpha
TRM	Transplant-related mortality
USA	United States of America
VAD	Vincristine-Adriamycin-Dexamethasone
VCD	Bortezomib-Cyclophosphamide-Dexamethasone
VGPR	Very good partial response
VMP	Bortezomib-Melphalan-Prednisone

VRD	Bortezomib-Lenalidomide-Dexamethasone
VTD	Bortezomib-Thalidomide-Dexamethasone
VTE	Venous thromboembolism
WBLD-CT	Whole-body low-dose computed tomography

CHAPTER 1: LITERATURE REVIEW

1.1. Introduction and epidemiology of multiple myeloma

Multiple Myeloma (MM) is a clonal plasma cell neoplasm that contributes to approximately 1% of all cancers worldwide and 10% of all haematological cancers. It is responsible for over 32 000 new cases and almost 13 000 deaths in the United States of America (USA) every year, with a yearly incidence of approximately 4 per 100 000 individuals.[1] The estimated incidence in Europe is slightly higher at 4.5-6 per 100 000 every year. The median age of patients at diagnosis is 65 years in the USA and 72 years in Europe. Males are afflicted by the disease slightly more commonly, with a male: female ratio of 1.6: 1 and black individuals have a two-fold increased prevalence compared to their white counterparts.[2]

According to the 2017 National Cancer Registry statistics of South Africa (SA), MM was responsible for 0.51% and 0.44% of all newly diagnosed cancers by histology in males and females, respectively.[3] A local study done in sub-Saharan Africa showed that the mean age at diagnosis was 61.4 years and that 7.1% of newly diagnosed MM patients were less than 40 years of age, thus highlighting the fact that patients are affected by MM at a younger age in our part of the world. A more recent study done at Chris Hani Baragwanath Academic Hospital (CHBAH) also showed a younger median age at diagnosis of 58 years.[4,5]

1.2. Pathogenesis of multiple myeloma

Multiple Myeloma occurs as a result of an unchecked proliferation of a clone of defective plasma cells in the bone marrow. Plasma cells are cells that live in the body for a long time and are secreted by immature, post-germinal centre B cells following exposure to foreign antigens found on the surface of bacteria and viruses. The plasma cells recognise subsequent infections with the same microorganisms and are responsible for antibody secretion and a faster and more effective immune response. Plasma cells form an important part of the body's humoral immune system and they are responsible for the secretion of multiple clones of immunoglobulins (Ig) in order

to carry out their function. Genetic aberrations during the production of plasma cells lead to the formation of a clone of faulty plasma cells, which leads to the uncontrolled secretion of an impaired monoclonal protein (or paraprotein).[6,7]

The most common subtype of MM is IgG kappa whereby the myeloma cells produce Ig consisting of 2 heavy IgG chains that are bound to two kappa light chains. 'Light chain' myeloma is a term used to describe a variant of MM in which the plasma cells secrete only the light chain portion of the Ig molecule. It is seen in approximately 20% of MM patients. These light chains are now measured by the serum free light chain (SFLC) assay, which has largely replaced urinary quantification of Bence Jones protein. About 2% of newly diagnosed MM patients present with non-secretory disease and have no evidence of the M protein using the common standard immunoassay techniques. [8,9]

The exact aetiopathogenesis of MM is unknown. Monoclonal gammopathy of undetermined significance (MGUS), a common premalignant condition present in 3.2 – 4% of the general population aged more than 50 years, has consistently been associated with progression to plasma cell neoplasms at a rate of about 1-2%/year. MGUS has a 20-year risk of transforming to MM of about 18%. There is an increased risk of first-degree relatives of patients with MM or MGUS developing MM in the future.[10]

Monoclonal gammopathy of unknown significance may progress to a more aggressive intermediate entity, although still asymptomatic, called smouldering multiple myeloma (SMM). The risk of transformation to MM is higher among patients with SMM as compared to MGUS, with the rate of transformation being about 10%/year in the first five years, then 3%/year over the following five years and finally 1.5%/year thereafter. Approximately 4100 individuals get diagnosed with SMM each year in the USA. MGUS and SMM are asymptomatic stages of monoclonal gammopathies and they do not demonstrate involvement of end organ damage.[10] Individuals who have been exposed to high levels of ionising radiation

(e.g., the Japanese people who were exposed to the two atomic bomb explosions in World War II), as well as to pesticides used in agriculture have been shown to display an increased risk of developing MM.[6]

1.3. Clinical presentation

The proliferation of abnormal plasma cells leads to end-organ damage. This forms the basis of myeloma-defining events (MDE) that are required, in addition to other criteria, to make the diagnosis of the disease. These MDE are usually abbreviated as the CRAB criteria (hyperCalcaemia, Renal insufficiency, Anaemia and Bone lesions), together with the three defining events mentioned further on page six, and their presence should only result from the abnormal plasma clone to be defined as MDE.[1,11]

The most common symptom of MM is bone pain and the most common clinical sign is anaemia. A haemoglobin concentration of < 12 g/dL is seen in up to 40–72% of newly diagnosed MM patients. Most patients display an anaemia of chronic disease associated with the high levels of cytokines, such as tumour necrosis factor α (TNFα) and interleukin (IL)-1, which suppress marrow production; a macrocytic anaemia is not unusually seen. In the presence of high concentrations of serum Ig, rouleaux formation is often present. The proliferation of defective plasma cells in the marrow causes a disruption in red cell production, which leads to the anaemia. The chemotherapeutic agents, such as the immunomodulatory drugs (IMiDs) and melphalan, that are used at different stages of MM therapy have potent myelosuppressive properties, which also contributes to the anaemia. Kidney disease, which is a MDE, is also involved in the pathogenesis of anaemia due to a deficient production of erythropoietin, which is a substrate required for the production of red blood cells.[8]

The osteolytic lesions seen in MM occur from an imbalance between the bone-resorbing osteoclasts, the bone-forming osteoblasts, bone marrow stromal cells, and osteocytes. Myeloma bone disease ultimately results in the destruction of the bony

elements and presents with significant bone pain, pathological fractures, and symptomatic hypercalcemia. The inevitable consequences of myeloma-associated bone disease and pathological fractures are a declining performance status and poor quality of life secondary to pain and hypercalcemia. Many factors contribute to the aetiology for the excessive bone mass loss seen in MM. Osteolytic lesions result from an imbalance between bone formation and bone destruction. In the bone marrow milieu, MM cells interact with bone marrow stromal cells (BMSCs) to cause an enhanced secretion of cytokines such as receptor activator of nuclear factor kappa B ligand (RANKL), macrophage inflammatory protein 1α (MIP-1α), IL-3 and IL-6. These cytokines increase osteoclastic function and bone resorption. On the other hand, a different group of cytokines activated by myeloma cells and BMSCs, such as Dickkopf-1 (DKK-1), soluble frizzled related proteins and sclerostin inhibit the WNT/bone morphogenetic signalling cascades that are important for maturation of mesenchymal stem cells into bone-laying osteoblasts.[12] The net result of this imbalance is that there is an increased risk of developing skeletal-related events (bone pain, pathological fractures of long bones, osteolytic bone lesions, vertebral compression fractures, osteoporosis and hypercalcaemia).[13]

Abnormalities of the bone architecture are seen in up to 80% of newly diagnosed MM patients. A study done in 2003 on a large patient population showed that 67% of the patients presented with lytic bone lesions and 20% showed features of osteoporosis complicated by pathological bone fractures at the time they were diagnosed with MM. Bone pain is the most common symptom in newly diagnosed MM patients. The risk of a MM patient developing a bone fracture at any point during the disease course has been demonstrated to be approximately 60%. The tumour load in MM is related to the severity of the bone disease. Prognosis is worse as the number of osteolytic lesions increases.[8,14]

Between 20-40% of MM patients have renal involvement at presentation, which impairs the survival rate as compared to MM patients without renal impairment. Kidney disease in MM can present in various ways, such as a light chain cast

nephropathy, monoclonal Ig deposition disease, or as AL amyloidosis. Cast nephropathy (myeloma kidney) is the most common renal complication of MM and is also considered a MDE. It is characterised by the formation of light chain casts within the renal tubular system, which cause obstruction in urinary flow downstream in the more distal collecting tubules. The severity of the renal impairment is directly related to the level of free light chains in serum or urine. Renal impairment in MM is also attributable to many other precipitants apart from the direct effects of myeloma protein. Many complications related to the disease itself, such as hypercalcemia, sepsis, uric acid nephropathy, dehydration and rhabdomyolysis as well as nephrotoxic agents such as contrast agents given during CT scan, nonsteroidal anti-inflammatory drugs (NSAIDs), and renin-angiotensin system antagonists are all implicated in the pathogenesis of nephropathy in MM. Bisphosphonates such as zoledronic acid given to myeloma patients can cause acute kidney injury in occasional instances. Pamidronate is another bisphosphonate that can cause a collapsing focal segmental glomerulosclerosis.[15,16]

1.4. Diagnosis of multiple myeloma

Serum protein electrophoresis (SPEP), immunofixation and the SFLC analysis are common laboratory techniques that are employed to demonstrate the presence of a serum paraprotein in a patient who is being worked up for MM. SPEP is useful to confirm the presence of a monoclonal protein. Immunofixation is useful as it increases the diagnostic sensitivity to 93% and helps to establish the subtype of the involved paraprotein (e.g., IgA, IgG or IgM myeloma) and if there is involvement of the kappa or lambda light chain. IgM multiple myeloma occurs rarely and its presence should make one think of the related disease Waldenström's macroglobulinaemia/lymphoplasmacytic lymphoma. MM may not be picked up by SPEP and immunofixation alone in a small contingent of patients with the light chain variant and would thus be missed and cause unnecessary delays in treatment. To avert this issue, an SFLC assay must always be requested. The sensitivity of diagnosing MM is as high as 97–98% when the above three tests are used together.[17]

The diagnosis of light chain myeloma has traditionally been more difficult as these low molecular weight proteins are quickly eliminated by the kidneys and 24-hour urine collections have their own technical difficulties, which causes irregularities in the result. Recent studies have demonstrated the superiority of the SFLC assay as compared to 24-hour urine assessments for identifying patients with light chain disease and for assessing response to initial therapy.[18] The International Myeloma Working Group (IMWG) has proposed the use of the SFLC in place of the more cumbersome and technically difficult 24-hour urine protein electrophoresis and immunofixation for future diagnostic purposes.[11]

Quantifying the amount of the monoclonal protein and SFLC prior to the initiation of therapy is necessary for demonstrating the magnitude of the disease at the start and to assess the response after therapy. An important aspect of treatment response in MM is to document an improvement in the levels of the serum paraprotein and SFLC over time.

Basic blood tests such as a full blood count (FBC) and film, kidney function and serum levels of calcium, magnesium and phosphate should always be done as part of the initial investigations in order to assess the extent and severity of MM. For the classification of MM into different severity categories, the guidelines suggest incorporating serum β2-microglobulin, albumin and lactate dehydrogenase (LDH), in addition to skeletal imaging modalities (skeletal survey) to detect and assess the extent of bone involvement by the disease.[11] Quantification of aberrant plasma cells and cytogenetics on bone marrow aspirate and trephine (BMAT) provide further information in terms of diagnosis and stratification of the disease into different risk categories, which are important determinants of treatment.

Any one or more of the MDE along with bone marrow evidence of at least 10% clonal plasma cells or a biopsy-proven plasmacytoma form the basis of diagnosis of MM as per the 2014 IMWG guidelines. Established CRAB features and the presence of three specific biomarkers, namely clonal bone marrow plasma cells (BMPC) ≥60%, SFLC

ratio ≥100 (provided involved SFLC level is ≥100 mg/l), and more than one focal lesion ≥ 5 mm in size on magnetic resonance imaging (MRI) constitute MDE.[11]
Evaluation of the bone marrow aspirate and/or trephine biopsy is essential to quantify and characterise the abnormal plasma cells in the bone marrow. Moreover, more specialised tests such as cytogenetic studies and fluorescent in situ hybridisation (FISH) are available to characterise the cytogenetic and karyotypic abnormalities that are usually displayed by the malignant plasma cells in the bone marrow sample; this has important implications for the patient in terms of risk stratification and guiding the most appropriate therapy.

Whole-body low-dose computed tomography (WBLD-CT) is now recommended as the new imaging modality of choice for the diagnosis of osteolytic lesions in MM. Plain radiographs are an alternative if WBLD-CT is accessible. The major limitation when using plain skeletal radiographs is that the pick-up rate for lytic lesions is only appreciated when at least 30-50% of the cortex is eroded and the patient has a high risk of developing pathological fractures. CT scan, as demonstrated in a study done on 212 patients, has proven to be superior to plain radiography at identifying lytic bone lesions in MM.[19] At facilities where MRI is available, it should be used in order to assess for bone lesions as it provides more information than other modalities and it is especially useful for assessing suspected spinal cord compression. Whole-body MRI or MRI of the spine and the pelvis are good imaging modalities, when available, to assess the BMPC infiltration, particularly the presence of bone focal lesions. MRI has a better sensitivity at picking up signal intensity changes, vertebral body compression fractures and soft tissue plasmacytomas. When available, 18F-fluorodeoxyglucose positron emission tomography with CT (PET-CT) is another imaging modality that provides valuable information in the assessment of bone lesions.[11]

1.5. Bone marrow and molecular studies

The accurate reporting of plasma cells in bone marrow is an important step in the diagnosis and assessment of treatment response in patients with MM. The IMWG

criteria define MM as the presence of ≥ 10% BMPC on aspirate or trephine biopsy specimens. Moreover, patients with ≥ 60% clonal BMPC should be treated as asymptomatic MM, even in the absence of MM-related organ damage. Complete response (CR) is defined as < 5% clonal plasma cells in the bone marrow as per the IMWG criteria. Accurate measurement of bone marrow plasma cells is therefore very important in order to assess response to therapy. Different factors, ranging from blood dilution, age- and therapy-related bone marrow hypoplasia as well as increased marrow fibrosis in MM, affect the quality of the bone marrow aspirate. Bone marrow trephine biopsies have been demonstrated to yield higher plasma cell numbers as compared to marrow aspirate samples. [7,20]

Flow cytometry is useful to identify and quantify neoplastic plasma cells as opposed to polyclonal plasma cells in the bone marrow. The immunophenotypic characteristics displayed by MM cells, irrespective of the different disease subgroups, are markedly different from those of normal plasma cells. Typically, CD38, CD138 and CD45 are immunophenotypic markers that are expressed more frequently and with higher intensity in neoplastic plasma cells as compared to other haematopoietic cells. Clonal plasma cells have been shown to display a different population of surface or intracellular antigens as compared to normal plasma cells, namely a weak expression of CD19, CD27, CD38, and/or CD45, an overexpression of CD28, CD33 and CD56, and an asynchronous expression of CD20, CD117, and/or surface Ig. Multiparameter flow cytometry has additionally been advocated as an important laboratory tool to assess for minimal residual disease (MRD) in patients who achieve deep remission status following therapy for MM. This has important prognostic significance in the era of novel therapies being used for MM treatment.[21]

Multiple myeloma is characterised by several cytogenetic abnormalities which are important prognostic factors. These gene alterations can be primary, disease-initiating anomalies that can be found as early as the development of MGUS (e.g., hyperdiploidy and IgH translocations) and secondary, as seen later in the course of the disease. FISH on interphase cells is run on the marrow sample to identify such

abnormalities.[6,12] Translocations involving the IgH locus on chromosome 14q32 are common, being present in almost 65% of MM patients. Hyperdiploid MM is a primary chromosomal abnormality that usually consists of trisomies of certain odd-numbered chromosomes with a few structural changes; it generally confers a good prognosis and is associated with a longer overall survival (OS). Trisomies confer varying prognostic outcomes {e.g., trisomy 21 impairs, while trisomies 3 and 5 improve, OS and may nullify the adverse impacts of del 17p and t(4;14) to some extent}. The presence of secondary cytogenetic abnormalities such as del 13q, del 17p, del 1p, or gain/amplification 1q and C-MYC translocations, along with the primary anomalies, adds to the burden of MM heterogeneity, which has important prognostic and therapeutic implications for the patient. These primary and secondary cytogenetic abnormalities are used in the R-ISS staging and risk stratification system of MM.[22] Deletion 17p is considered the most detrimental prognostic factor and is associated with a short remission after high-dose therapy (HDT) and an increased incidence of extramedullary disease. It is seen in about 8-10% of untreated patients and has phenotypic implications when present in most plasma cells.[23]

1.6. Prognosis and risk stratification

Multiple myeloma is a heterogeneous condition with variable outcomes with or without treatment. This is largely a result of various prognostic factors involved in the pathogenesis of the condition. A number of factors, namely the biological characteristics of the disease, global disease burden and the performance status of the patient determine how every patient responds to the disease and to the treatment thereof. Cytogenetic abnormalities detected by FISH have a profound effect on the way the disease behaves and subsequently responds to chemotherapy. High-risk MM is defined by the presence of t(4;14), t(14:16), t(14;20), deletion 17p, TP53 mutation or gain/amplification 1q.[9,24] Double and triple hit MM refer to the presence of two high-risk features and ≥ three high-risk features, respectively. Extramedullary disease and plasma cell leukaemia are regarded as more aggressive forms of MM that require as extensive treatment as high-risk MM.[25]

Different clinical scoring systems have been put in place to estimate individual prognosis. The Durie-Salmon staging system was used in clinical trials for many years to provide standardisation and included important prognostic parameters such as the severity of anaemia, renal failure and lytic bone lesions.[26] This was replaced by the more recent International Staging System (ISS), which incorporated two biomarkers related to tumour burden and disease severity, namely serum β2-microglobulin and albumin, in 2005. The ISS, a powerful and reproducible three-stage classification, was derived from a large study across three continents and subsequently validated to be used as a more reliable staging system globally and equally among younger patients < 65 years old as well as their older counterparts. Stage I was associated with a median survival of 62 months, stage II 44 months, and stage III 29 months, respectively.[27] These two stratification systems were subsequently superseded by the Revised International Staging System (R-ISS), which added 3 cytogenetic risk factors [del(17p), t(4;14), t(14;16)] and serum LDH levels to the ISS. To ensure uniformity, the R-ISS uses only 3 of the widely available cytogenetic markers for risk assessment. R-ISS stage III, which is associated with the most adverse prognostic parameters, has a median PFS of only 29 months as compared to R-ISS I, which is related to a median PFS of 66 months.[24] The Mayo Stratification of Myeloma and Risk Adapted Therapy (mSMART) consensus guidelines make use of additional cytogenetic abnormalities and other markers [e.g., t(11;14), t(6;14) and hyperdiploidy, to name a few] for risk stratification. The ISS and R-ISS stratification systems, unlike their importance in determining prognosis in newly diagnosed MM, have not been validated for use in the setting of relapsed/refractory MM.[9]

Patient-related factors that can influence prognosis include age, frailty, performance status (PS) and co-morbidities, among other factors. High-risk MM patients have a more aggressive disease course resulting in poorer outcomes than their counterparts with standard-risk disease. These patients therefore benefit from more aggressive intensive chemotherapy regimens and maintenance therapies for them to achieve the desired disease response. Therefore, risk stratification is an important tool in MM

staging in order to determine the most appropriate treatment approach for each patient.[23]

1.7. Management

Young, fit, "transplant-eligible" patients (< 65 years) are offered a combination of highly effective induction chemotherapeutic agents, which elicit quick and deep responses, for at least 4-6 cycles in order to achieve a sustained CR and long-term disease control. Ideally, patients who obtain a good response, that is CR or very good partial response (VGPR), should be offered consolidation therapy with HDT and autologous stem cell transplantation (ASCT). This is followed by maintenance therapy with 1-2 agents in order to sustain the remission status of the patient and to reduce the risk of disease relapse for as long as possible.

1.7.1. Supportive measures

Supportive therapy, along with specific MM therapy, is important in improving the quality of life of the patient and consists of treating the complications related to MM itself or the different treatment modalities used to treat the condition.

Bisphosphonates have consistently been shown to improve the outcome of bone disease when used as supportive therapy in MM. Bisphosphonates, such as zoledronic acid and pamidronate, bring about a downregulation in osteoclastic activity. Bisphosphonates have been shown in many trials to improve a number of parameters in bone-related disease, namely a decreased incidence of pathological fractures, an improvement in bone pain and fewer skeletal-related events. However, data regarding whether these agents also improve survival in MM are not conclusive.[28] Pamidronate is given as a 90 mg intravenous infusion over 3 hours once monthly. Zoledronic acid 4 mg is given intravenously over 15 minutes once monthly. When choosing a particular bisphosphonate, one should take into consideration the patient's presentation, the renal function, and the side effects/complications that are associated with each agent. For example, zoledronic acid is very effective in the treatment of hypercalcemia and pamidronate is a safer agent in patients with severe

renal disease. One study showed that addition of zoledronic acid to standard first-line anti-myeloma treatment decreased the risk of dying by as much as 16% as well as having a favourable impact on both OS and PFS.[28] The most important side effects of bisphosphonates include osteonecrosis of the jaw, atypical fractures, and acute kidney injury (specific to zoledronic acid).[28] A reduction in the frequency of bisphosphonate administration, down to 3- to 6-monthly, can be considered by the treating physician depending on the risk profile for osteoporosis in the given patient. The latest guidelines suggest at least a VGPR before the dosing interval of bisphosphonates can be increased. Patients who have not achieved at least a VGPR should continue to receive bisphosphonate therapy monthly. It is recommended to re-initiate bisphosphonate therapy when there are signs of clinical or biochemical relapse of MM.[29]

Denosumab is a RANKL inhibitor which suppresses osteoclastic activity, improves bone density and reduces the incidence of osteolytic lesions in MM. It is used as a second line therapy, when bisphosphonates are contraindicated or ineffective, for the treatment and prevention of osteolytic lesions in MM. Recently, a large phase III randomized controlled trial conducted on 1,718 newly diagnosed MM patients confirmed that denosumab 120 mg given subcutaneously every four weeks was non-inferior to a 4-weekly regimen of zoledronic acid 4 mg given intravenously in delaying the occurrence of skeletal-related events. The OS was comparable in both treatment arms. Furthermore, unlike bisphosphonates, denosumab is cleared from the body via the reticuloendothelial system rather than the kidneys and it is not known to cause any nephrotoxicity. It is thus a more suitable agent than bisphosphonates in MM patients with significant renal dysfunction.[13]

Different treatment modalities are available to treat bone pain in myeloma-related bone disease. Different classes of analgesic medications, when used along with anti-myeloma therapy, are preferred as the first-line treatment. Painful vertebral compression fractures can be treated effectively with kyphoplasty.[30] If the above are not successful or technically not possible, the patient can be offered palliative

radiation therapy, which has shown benefit in treating very painful lytic lesions or plasmacytomas not responding to conservative measures and systemic anti-myeloma therapy. Palliative pain relief is usually obtained with radiation doses of 20-30 Grays given in 5-10 fractions.[31]

Hypercalcemia is treated with a combination of aggressive intravenous fluids, corticosteroids, bisphosphonates, mobilisation and anti-myeloma therapy. Severe, refractory cases of hypercalcemia may require haemodialysis.

Aggressive hydration in patients with no contraindications (e.g., heart failure) and fast-acting chemotherapeutic agents are essential first-line modalities to decrease the extent of kidney damage in MM. For example, a bortezomib (Velcade)-based triplet therapy, such as bortezomib, cyclophosphamide, and dexamethasone (VCD), is an effective regimen for patients presenting with MM and acute kidney injury (AKI), as bortezomib does not affect renal function. Particular attention should be given to the control of hypertension, adjusting the doses of, or stopping nephrotoxic drugs (NSAIDs, chemotherapy agents, antibiotics) based on the creatinine clearance,[15] and avoidance of contrast agents and dehydration as best as possible in order to mitigate further damage to the kidneys.

Supportive management of anaemia in MM can be managed by supplementation with haematinic products if they are deficient or by transfusion of red blood cells. The use of erythropoietin-stimulating agents such as darbopoetin alfa and epoetin alfa to improve anaemia in cancer patients, including MM, is no longer recommended as these agents have consistently been shown to increase the risk of thromboembolic events in these patients.[32] These agents should therefore be used with caution in MM patients.

Peripheral neuropathy usually occurs as a result of the disease itself or due to treatment-related toxicity. IgM monoclonal gammopathy is the most common subtype related to peripheral neuropathy. The exact mechanism is poorly understood

and is likely related to the adverse effects of the paraprotein on myelination of peripheral nerves. Chemotherapy agents, particularly bortezomib and thalidomide, are associated with high rates of peripheral neuropathy. Bortezomib is associated with a particularly painful and debilitating neuropathy, occurring in up to 60% of patients receiving a twice-weekly dose. Using a once-weekly dose and administration through the subcutaneous rather than the intravenous route both reduce the incidence and severity of neuropathy associated with bortezomib. Furthermore, a range of neuropathic pain medications, such as duloxetine, tricyclic antidepressants and anticonvulsants, can be given to alleviate severe debilitating pain.[1,11,33]

Patients with MM are at high risk of developing infections, including severe life-threatening infections. The risk of death from infections is especially high in the first three months of the disease. Multiple factors, including dysfunctional plasma cells, the use of corticosteroids, immune-paresis and treatment-related neutropenia account for this heightened risk. Infection prophylaxis forms a regular and major part of the treatment plan for any MM patient. MM patients should have yearly flu vaccines and also a once-ff pneumococcal vaccine at diagnosis in order to reduce the risk of infections.[34] Myeloid growth factors such as granulocyte colony stimulating factor (G-CSF) should be used during treatment, especially to improve neutrophil counts after chemotherapy agents cause bone marrow suppression, and as supportive treatment in relapsed disease.

The risk of developing venous thromboembolism (VTE) at any point during the course of MM is reported to be more than 10%. There are many risk factors, mostly working in synergy, that increase the thrombogenic potential in MM patients. Multiple guidelines have devised a risk stratification algorithm and choice of thromboprophylaxis based on patient-related, disease-related and therapy-related factors. The mechanisms underlying the development of thrombosis are still poorly understood to this date. A new diagnosis of MM itself is a major risk factor due to the extensive burden of the disease and its relationship with release of pro-inflammatory and procoagulant cytokines. Platelet dysfunction and increased

adhesion is another characteristic of the disease early on. The immunomodulatory drugs (IMiDs) thalidomide, lenalidomide and pomalidomide are associated with an increased frequency of thromboembolism, especially when used with high dose dexamethasone or as part of a multidrug regimen. The exact mechanism of causing thrombosis with these agents is still not fully understood but is thought to be related to increased activity of the procoagulants von Willebrand factor, factor VIII and tissue factor.[35]

In terms of therapy, the current IMWG and National Comprehensive Cancer Network (NCCN) guidelines recommend using aspirin as thromboprophylaxis for all MM patients, except patients with at least two risk factors who need to be treated with prophylactic doses of low molecular weight heparin or therapeutic doses of warfarin.[35,36]

Direct oral anticoagulants (DOAC), which are direct inhibitors of FXa or FII in the coagulation cascade, have recently been approved for treating established thrombotic events in cancer patients. The advantages with these agents are that they are taken orally and do not need regular monitoring of blood levels. Their role in thromboprophylaxis in MM patients is yet to be established while awaiting the final results of promising prospective trials, although they are being increasingly used by cancer institutions around the world.[36]

1.7.2. Induction chemotherapy

The choice of induction agents in the treatment of newly diagnosed MM depends on whether the patient is an ASCT candidate or not. Before the introduction of the novel agents, conventional chemotherapy options such as melphalan-prednisone (MP) or vincristine-adriamycin-dexamethasone (VAD) were not ideal in achieving satisfactory responses. MP and VAD were the commonly available options across centres and were used with the goal of achieving a partial response (PR) or disease stabilisation. Only a few patients achieved a durable CR and the expected OS was dismal, at approximately 30 months.[37] Furthermore, these agents could not be used

for prolonged periods due to their cumulative chemotherapy toxicity and the risk of stem cell damage, thereby increasing the risk of developing leukaemia.

In transplant-eligible candidates, three or more cycles of induction chemotherapy are given to achieve an adequate response prior to stem cell harvest. Proteasome inhibitors (PIs) and IMiDs are novel chemotherapy agents that have helped to shape the landscape of MM therapy over the last 10 years. Bortezomib, first approved for use in the treatment of MM in 2003, reversibly inhibits the proteasomes in aberrant plasma cells and therefore leads to their apoptosis. The PIs and IMiDs (thalidomide and lenalidomide) are highly effective agents against MM cells when used together and have therefore evolved as the most commonly used drugs to form part of induction regimens in many centres worldwide. The new triplet regimen consisting of bortezomib, lenalidomide (Revlimid) and dexamethasone (VRD) is the current standard of care option for newly diagnosed MM due to its improved OS and PFS demonstrated in a number of studies.[38,39,40] The Southwest Oncology Group (SWOG) trial showed a clear benefit when using the VRD regimen as compared to a lenalidomide-dexamethasone (RD) regimen in terms of median PFS, OS and overall response.

The triplet regimen consisting of bortezomib, cyclophosphamide and dexamethasone (VCD) has also proven to be safe and effective in newly diagnosed MM patients undergoing ASCT, especially in patients with renal insufficiency, where lenalidomide cannot be used due to its renal excretion.[41] The thalidomide-based triplet regimen VTD (bortezomib-thalidomide-dexamethasone) can also be considered an alternative to VRD for initial therapy in patients with renal insufficiency, or in settings where lenalidomide is not readily accessible. The novel agents have clearly been shown to improve survival in newly diagnosed MM patients but they are sometimes limited by their side effect profiles. Lenalidomide is excreted by the kidneys and is therefore not recommended in patients with pre-existing renal impairment. It has also been associated with an increased incidence of venous thromboembolism. This risk is especially high when lenalidomide is used in combination with high-dose

dexamethasone. It is therefore recommended that lenalidomide be combined with a lower dose of dexamethasone (40 mg once weekly) to reduce this risk and, in patients with a high risk for VTE, prophylaxis with a low molecular weight heparin or warfarin be used.[42] The same principle applies to patients on thalidomide-based regimens.[35] Finally, lenalidomide therapy has been found to negatively impact collection of peripheral blood stem cells (PBSC) for ASCT. A longer course of induction with lenalidomide further reduces the yield of PBSC. Patients who have received prolonged therapy with lenalidomide might benefit from plerixafor, a chemokine receptor 4 inhibitor, to improve stem cell mobilization from the bone marrow.[42] Bortezomib is well recognised to cause peripheral neuropathy. This risk is especially significant when the agent is used as a twice weekly dose and when used as an intravenous formulation. The risk can be decreased significantly when bortezomib is used once weekly instead of the usual twice weekly dose and when it is given via the subcutaneous route as compared to the intravenous route that is commonly used in clinical trials.[38]

Daratumumab, a human IgG1 kappa monoclonal antibody that targets CD38, has direct antitumor and immunomodulatory activity. Recently, a daratumumab-based regimen, namely daratumumab, lenalidomide (Revlimid) and low-dose dexamethasone (DRd) has shown improved PFS compared to lenalidomide and low-dose dexamethasone (Rd) in a randomized trial conducted in transplant-ineligible patients.[43] In patients with pre-existing neuropathy or who are intolerant to VRD, DRD is increasingly recognised as a suitable alternative. In high-risk patients, especially those with double-hit or triple-hit MM, a daratumumab-based quadruplet regimen (Dara-VRD) is now considered the new standard induction treatment.[41] The use of daratumumab in induction chemotherapy regimens has thus expanded in the past few years to include both newly diagnosed and refractory/relapsed MM, and for transplant-eligible and ineligible patients.

There are multiple chemotherapy regimens approved for use in patients who are deemed unfit to undergo ASCT or those who opt out of the procedure. The available

options in such patients usually consist of a three-drug combination regimen, such as DRD or VRD. This is then followed by lenalidomide maintenance therapy. VRd was shown to be superior to Rd in patients older than 65 years in the SWOG trial.[38] Lenalidomide or bortezomib combined with a glucocorticoid has been used as a safer option in treating older patients who are unable to tolerate the side effects related to VRD.

Melphalan is a potent antineoplastic agent and has been found to cause stem cell damage, secondary myelodysplastic syndrome and acute leukaemia. Hence, the use of melphalan as an induction agent is no longer common practice. VRD is generally given for approximately 8–12 cycles, followed by maintenance therapy. In order to minimise their side effects, lenalidomide and dexamethasone are administered in lower doses to frail elderly MM patients. If therapy with VRD is not possible due to inability to travel for intravenous administration, ixazomib can be considered as an alternative to bortezomib. DRD has been found to be associated with a significantly superior PFS and a higher MRD-negativity rate when compared to Rd in a randomised control trial done in the USA. It was subsequently approved in the USA for use as an alternative option in newly diagnosed MM patients who are transplant-ineligible and have any contraindication to VRD. As opposed to VRD, which is used for only a limited period of time (8-12 months), the main concern when using DRD is that the regimen is given until signs of disease progression appear. This might be for many months to years, implying higher costs related to this regimen and more treatment-related complications for the patient.[43]

1.7.3. High-dose therapy/Autologous stem cell transplantation

High-dose therapy followed by ASCT has superseded conventional chemotherapy as the current standard treatment for newly diagnosed MM patients since the two seminal studies done in the 1980s, confirming the superiority of high-dose melphalan and ASCT in achieving better outcomes.[44,45] Another groundbreaking study by Attal and colleagues 25 years ago and done before the advent of novel agents showed a clear benefit of upfront ASCT in terms of PFS and OS at the time of analysis. Median

survival of patients prior to that study was up to three years.[23] Multiple randomised trials involving novel agents over recent years have demonstrated continued benefits of ASCT. The long-held belief that early ASCT was associated with better outcomes still applies to some extent in the era of novel agents. Longer PFS, treatment-free intervals and better quality of life are all associated with the use of early ASCT. These benefits, however, did not translate into higher OS benefit when compared to delayed transplantation given at disease relapse. Thus, delayed ASCT is still a safe alternative in the current MM treatment landscape of novel therapies.[28]

A cut-off age of 65 years has traditionally been used as an entry criterion for stem cell transplantation in most clinical trials. Recent studies have shown a significant benefit of ASCT in patients at least 70 years old with minimal comorbidities and a good PS. These patients achieved similar anti-myeloma benefits without an increase in transplant-related mortality (TRM) and relapse rate and had improved PFS as compared to their younger-aged counterparts. These findings have led to many transplant centres reviewing their age cut-offs and including select older patients as part of their treatment algorithms.[13]

The constantly evolving armamentarium of novel anti-myeloma chemotherapeutic agents has led many clinicians to question the role of HDT–ASCT as consolidation due to the severe toxicity related to high-dose melphalan and the increasingly promising results being seen with the newer agents. Although TRM is very low in experienced centres (< 1%), high-dose melphalan, owing to its alkylating agent effect, has been linked to the development of secondary malignancies later in life and can potentially cause genomic aberrations.[46] With novel agents being more regularly used across most centres now, many clinicians are looking into new treatment strategies for young MM patients and finding alternatives to replace HDT–ASCT with a non-transplant approach. There are, however, high-powered studies demonstrating a clear beneficial role of ASCT in the era of novel agents. The EMN02/HOVON95 phase III study showed a clear benefit in terms of the median PFS (57 versus 41 months) of HDT-ASCT when compared to a consolidation

regimen consisting of four cycles of bortezomib–melphalan–prednisone (VMP) in a large cohort consisting of 1,503 patients.[47] These convincing results in favour of ASCT over consolidation therapy with novel agents were further supported by the IFM 2009 study.[40]

1.7.4. ASCT Eligibility

With regards to an age limit beyond which ASCT is deemed not to be safe or effective, there is still ongoing debate and no general agreement exists among various transplant institutions and countries across the world. In order to limit the complications and deaths related to HDT/ASCT and withdrawal from studies, most clinical trials recruit patients who are ≤ 65 years for them to be transplant-eligible. This is the case in most transplant centres across Europe, where older patients generally do not benefit from an ASCT. On the other hand, the growing trend in American clinical trials has been to include a wider number of newly diagnosed MM patients up to 75 years of age with good PS and minimal comorbidities to receive an ASCT. A recent analysis of both the European Blood and Marrow Transplantation (EBMT) and Centre for International Blood and Marrow Research registries clearly showed a constant increase, from 1991-1995 to 2010, in the use of ASCT in older patients (> 65 years).[48] A prospective study done on 102 patients and including patients up to 75 years showed that HDT with melphalan 100 mg/m^2 followed by ASCT was both safe and effective (5-year OS, 63%) in patients with a good PS and no comorbidities. There was no direct correlation between age and PFS in this cohort. The younger age group (66-70 years) also had a lower incidence of TRM than patients older than 70 years (5% vs 19%).[49] Another relevant study that compared melphalan 140 mg/m^2 with melphalan 200 mg/m^2 in patients > 65 years found that the TRM rate more than 100 days after ASCT was 0% in both subgroups.[50] These two studies have clearly demonstrated that the use of high-dose melphalan as a conditioning regimen is a relatively safe modality in older patients > 65 years. There has been a notable improvement in both the PFS and OS in “elderly” patients after 2008, due to the more regular use of novel agents in the treatment algorithms. This has led to an interesting debate among experts who argue that the chronological age

of patients in the era of novel agents does not significantly impact on the treatment outcome when they are subjected to an ASCT, as long as these patients are carefully selected.[51] Therefore, the actual age of the patient should not be used as the sole factor to determine the eligibility for ASCT. The organ function of the patient, associated comorbidities and the performance status should all be taken into account to determine whether the patient may undergo an ASCT. New evidence in the United States is showing that ASCT can safely be offered to select patients up to the age of 80 years.[52]

1.7.5. Mobilisation of bone marrow stem cells

Apheresis of an adequate number of haematopoietic stem cells from peripheral blood that can be used for a successful ASCT first involves using a mobilisation agent that can maximise the yield of $CD34^+$ cells from the marrow and subsequently harvesting those cells from the peripheral blood. At least 2×10^6 $CD34^+$ cells/kg are deemed to be acceptable for most patients who require at least one ASCT but, whenever possible, the goal stem cell collection amount for most patients in preparation for a potential second ASCT should be about 5×10^6 $CD34^+$ cells/kg or more. Various mobilisation agents are available, including chemotherapy mobilisation, growth factor–only mobilisation, and growth factor plus plerixafor mobilisation. There is good evidence to show that chemotherapy mobilisation, usually with cyclophosphamide (3-4 g/m^2) alone or combined with growth factors, or etoposide combined with growth factors, leads to a more favourable stem cell yield when compared to mobilisation with growth factor alone. This, however, comes at the cost of more infections and hospitalizations, and a higher need for blood products. Plerixafor is a chemokine receptor 4 (CXCR4) antagonist that impairs CXCR4 binding with the stromal derived factor 1 (SDF-1). The advantages of using plerixafor are numerous, including an enhanced mobilisation of $CD34^+$ cells into peripheral blood with a more prolonged effect and releasing a greater number of cells in the growth phase, thereby optimising the probability of a successful engraftment and immune reconstitution.[53] However, the high/exorbitant cost of plerixafor limits its use.

Chemo-mobilization is useful in patients in whom the MM tumour burden needs to be decreased significantly and to enhance stem cell mobilisation. However, chemo-mobilization is associated with more bone marrow toxicity as compared to mobilisation with G-CSF alone and there is evidence that chemo-mobilisation on its own is not related to superior OS outcomes when compared to other mobilisation agents.[53] Nonetheless, cyclophosphamide is an excellent chemo-mobilisation agent that is often used when G-CSF and/or plerixafor fail to mobilise enough stem cells, or in those patients who have progressive disease at the time of stem cell mobilisation. When the bone marrow is exposed to the anti-neoplastic effects of lenalidomide for a prolonged time, stem cell mobilisation is significantly impaired, which may render ASCT less effective. Evidence has shown that using plerixafor and/or chemo-mobilisation with cyclophosphamide and G-CSF during the first few cycles of lenalidomide-based induction chemotherapy is able to counter the negative impact that lenalidomide has on stem cell mobilisation.[54] It is therefore common practice to mobilise and collect stem cells within the first four to six cycles of lenalidomide-based induction therapy in order to obtain a better stem cell yield.

1.7.6. Conditioning regimen

The current accepted standard for HDT is intravenous high-dose melphalan (200 mg/m^2) and studies have shown that this dose is associated with the optimal balance between efficacy and toxicity. This agent is administered either as a single intravenous infusion lasting 30 to 60 minutes or as two infusions of 100 mg/m^2/day during a 2-day period, with forced diuresis. Other dosages, such as 140 mg/m^2 and 100 mg/m^2, showed a reduction in efficacy in terms of PFS and median time to progression. Persistent renal impairment or dialysis are not contraindications to HDT and ASCT. Administration of reduced doses of melphalan such as 140 mg/m^2 should be considered for patients over 70 years of age and patients who are dialysis-dependent or have a significantly impaired creatinine clearance of <30 mL/min.[55] Severe and prolonged myelosuppression is the major side effect of high-dose melphalan, which is the reason why ASCT is performed to reduce the duration of cytopenias. With the use of the patient's own mobilised peripheral blood stem cells,

the median duration of severe neutropenia and thrombocytopenia is seven days. Several studies evaluated the addition of other agents to melphalan. A randomized, single-centre, phase III trial showed that a conditioning regimen of Busulfan/Melphalan was safe and associated with an improved PFS (65 months versus 34 months) when compared with Melphalan alone. The 3-year OS was 91% with Busulfan/Melphalan and 89% with Melphalan. Therefore, this combination regimen may become the new standard of care in the near future.[56]

1.7.7. Timing of ASCT

There are many trials that have consistently shown that ASCT confers a survival benefit when compared to other treatment alternatives. However, there is still ongoing controversy when it comes to the optimal timing of ASCT for it to offer the best survival benefit to the patient. Early transplant versus late transplant was an important question even before we had novel therapies. The 1998 study by Fermand et al. showed improved PFS in early versus late ASCT in newly diagnosed MM patients, but there was no difference in OS between the two groups. Patients who were randomized into the early group received ASCT right away and those in the late group received conventional chemotherapy until progression or relapse when they were undergoing autologous transplantation. The authors also demonstrated that early ASCT provided longer event-free survival (EFS), suggesting a clinical benefit of early versus late transplantation.[57] Results of the IFM 2009 trial prospectively confirmed these findings by showing that, with the use of VRD induction, upfront ASCT improved median PFS (50 versus 36 months), but had a similar 4-year OS compared with ASCT upon experience of first relapse.[40] A pooled analysis of the GIMEMA-RV-MM-PI-209 and RVMM- EMN-441 studies showed improved PFS (71% versus 54% at four years), and OS (84% versus 70% at four years) in patients receiving HDT–ASCT upfront versus patients treated with novel agent triplet regimens without ASCT.[58] Nonetheless, evidence obtained from recent trials whereby patients received a bortezomib-based induction regimen did not show an improvement in survival benefit in patients who subsequently received HDT–ASCT

as compared to their counterparts who did not have an ASCT, suggesting that delaying ASCT at first relapse is a viable option.[40,47]

When deciding on the timing of the ASCT, there are a number of factors that have to be taken into account, e.g., standard-risk versus high-risk disease, the age of the patient, any comorbidities, the performance status of the patient, and the aggressiveness with which the disease is developing. A retrospective study showed that the probability of the patient becoming transplant-ineligible when opting for a delayed ASCT approach was approximately 12% due to a decline in the patient's performance status, more comorbidities and a higher risk of aggressive disease relapse as the patient waited longer to receive the treatment.[59] Patients with high-risk MM have more aggressive disease, more rapid progression and more complications as compared to patients with standard-risk MM. These patients therefore benefit enormously from early upfront ASCT, ideally within 12 months of diagnosis.[60] Upfront ASCT is associated with a higher probability of achieving a deep hematologic response, especially MRD-negative status, which is associated with improved PFS and OS.[61] Transplant facilities should therefore offer upfront ASCT to all high-risk patients who are eligible for ASCT in order to achieve the best outcomes. In any case, stem cells should be collected within 4 to 6 months of diagnosis in all transplant-eligible patients.

1.7.8. Single versus tandem transplant

A tandem ASCT is defined as a pre-planned second ASCT within 2-3 months of the first ASCT. Tandem transplants have been offered based on the assumption that they deepen the haematological response and hence improve outcomes conferred by a first ASCT. The results of trials to confirm this assumption have not been entirely convincing so far. For example, tandem ASCT was not found to improve the PFS and OS as compared to single ASCT in a meta-analysis that was done on studies before the introduction of the novel agents.[62] On the other hand, the HOVON trial, which was carried out in the era of novel induction agents, showed that tandem ASCT improved both the 5-year PFS (53.5% versus 44.9%, HR 0.74) and OS (80.3% versus

72.6%, HR 0.62) when compared to single ASCT. The greatest benefit of double ASCT as compared to single ASCT was appreciated in patients who had at least one of the adverse cytogenetic profiles, particularly del 17p. The risk of dying was decreased in patients with high-risk cytogenetics who received a tandem ASCT (HR 0.52).[47]

The role of tandem ASCT in the current era of novel agents is not well established in newly diagnosed MM patients. The commonly used induction regimens nowadays combine both a PI and an IMiD (mainly VRD), which result in deeper hematologic responses and PFS when combined with ASCT. The phase III BMT-CTN 0702 STAMINA trial that was conducted in the USA demonstrated that a tandem ASCT, when compared to either consolidation with four cycles of VRD or only lenalidomide maintenance after the first ASCT, did not improve the depth of response, PFS, or OS. All these patients had received induction chemotherapy with VRD prior to randomisation. Patients with high-risk cytogenetics did not gain a PFS or OS advantage with the use of tandem ASCT.[63] There is a good chance that the use of two novel agents in induction therapy curbs down the need for a tandem ASCT. This applies to patients with high-risk cytogenetics as well. If we take into consideration the conflicting results published so far, a second ASCT appears to be a feasible and reasonable option, especially for patients with high-risk MM and those who fail to achieve at least a VGPR after the first transplant.[47,63]

1.7.9. Salvage ASCT

The majority of patients will experience relapsed MM, despite consolidation and/or maintenance therapy after their first ASCT. There is no single established treatment option in the setting of relapsed MM and different institutions opt for different regimens, including allogeneic stem cell transplantation, combination chemotherapy with an IMiD and a PI and a second ASCT in patients with adequate stem cells. Novel agents used to treat relapsed MM, despite showing a modest survival benefit, are subject to high levels of chemotherapy resistance which ultimately leads to refractory disease in patients.[64] Two important retrospective studies have shown that salvage

ASCT to treat relapsed/progressive MM after a first ASCT as an attractive option as it is related to an improved PFS and OS and is related to very low non-transplant mortality rates. The first study analysed a total of 187 patients with relapsed MM and who were offered a salvage ASCT from various centres across North America. The study showed that, at five years after receiving a salvage ASCT, 29% of the patients were still alive and 25% of the patients were still showing a CR.[64] Similar outcomes were noted from the second study in which the median OS was four years after salvage ASCT and the median PFS was 18 months.[65] The mortality rates related to the salvage ASCT were very low in both these studies and the longer duration of response after the first ASCT translated into an improvement in both the PFS and OS after salvage ASCT as opposed to patients with a shorter duration of response in both of these studies. In order to further improve the outcomes after salvage ASCT, appropriate maintenance therapy should be offered to relapsed patients.[65] Therefore, salvage ASCT is most effective in patients who had a longer duration of response after their first ASCT, a good baseline PS and who have access to novel agents to be used for maintenance therapy.

1.7.10. Maintenance chemotherapy following ASCT

Post-transplantation maintenance therapy currently involves the use of 1-2 novel agents at a lower dose than that used for induction with the goal of maintaining the depth of response achieved by ASCT. The IMiDs thalidomide and lenalidomide often form part of maintenance regimens. In a large meta-analysis looking at over two thousand patients, thalidomide maintenance therapy was found to impart a significant OS benefit. On the contrary, the OS outcomes were worse when thalidomide was given to patients with high-risk cytogenetics, reflecting that thalidomide maintenance might play a role in favouring the selection of tumour cells and causing proliferation of disease in patients with high-risk cytogenetics. The major side effect of thalidomide maintenance, despite improving the disease control, was found to be peripheral neuropathy, which adversely impacted on the patients' quality of life.[66] Thus, when used in the maintenance setting, the dosage and duration should be limited to 100 mg daily and 6–12 months, respectively. Maintenance therapy with

lenalidomide is better tolerated than thalidomide. A meta-analysis of three randomised controlled trials and looking at 1,208 patients showed a significantly better median PFS (52.8 months versus 23.5 months) in the lenalidomide maintenance group as compared to the placebo/observation group. At a median follow-up time of 79.5 months for all surviving patients, median OS had not been reached for the lenalidomide maintenance group, whereas it was 86 months for the placebo group. This represented a 25% decrease in the incidence of death and an improvement in the median survival by approximately 2.4 years in the lenalidomide group as compared to the placebo group. The greatest OS benefit was seen in patients who had received lenalidomide as an induction agent. Lenalidomide maintenance was not related to resistant disease when the patient relapsed. In patients with high-risk cytogenetics, lenalidomide maintenance was associated with an improvement in PFS but not OS. Lenalidomide maintenance increased the cumulative incidence rate of secondary primary malignancies compared with placebo.[67]

1.8. Relapsed MM

Relapse is an imminent feature affecting almost all MM patients. With the use of novel agents, the first relapse of MM is seen after approximately 3–4 years following the initial diagnosis. Following a first relapse, each subsequent remission lasts for a shorter period after each new regimen is used to treat the relapse. A number of factors determine the choice of treatment after each relapse. These include the timing of the relapse, the response to prior therapy, the severity of the relapse, and the PS of the patient. Patients who are eligible for ASCT should be considered for ASCT if they had elected to delay the procedure, or if they achieved an excellent response duration with the first ASCT, defined as a remission of 36 months or longer with maintenance therapy. In general, a triplet regimen is preferred. At each relapse, a regimen that contains at least two new drugs that the patient is not refractory to should be considered. Treatment for relapsed MM is typically continued until disease progression.[68]

Patients who experience a first relapse and are not refractory to lenalidomide can be given one of several triplet regimens containing lenalidomide to achieve quick haematological responses. Each of these regimens has shown superiority over RD in randomized trials. Daratumumab can also be used as a subcutaneous formulation, which reduces infusion-related side effects and reduces the time for administration. Pomalidomide- or bortezomib-based combination regimens should be considered for induction in patients with a first relapse and who have been shown to be refractory to lenalidomide-based therapy. These combinations include daratumumab, bortezomib, dexamethasone (DVD), VCD, and bortezomib, pomalidomide, dexamethasone.[68]

Although patients who are refractory to a drug are likely to be refractory to a different drug in the same class, two important exceptions do exist. Pomalidomide has clinical activity in patients who are refractory to lenalidomide, and carfilzomib has activity in patients who are refractory to bortezomib.[68]

Venetoclax is an antineoplastic agent that has activity against B-cell lymphoma-2 (BCL-2) expression. BCL-2 expression has been shown to promote tumour formation and it also helps different tumours to evade apoptosis by anti-tumour cells. MM patients with t(11;14) express high levels of BCL-2. Venetoclax appears to have single-agent activity in MM patients with t(11;14). However, the results of a recent randomized trial found significantly higher mortality with venetoclax in relapsed MM despite producing deeper responses and better PFS. Therefore, the use of venetoclax should be restricted to patients with t(11;14) who have relapsed disease.[68]

1.9. HIV and MM

There is a paucity of data concerning ASCT in human immunodeficiency virus (HIV)-positive patients with MM in the pre-combination antiretroviral treatment (cART) era because of difficulties related to management of treatment-related complications for patients with HIV. MM is less commonly associated with HIV than is non-Hodgkin lymphoma. HIV has been found to be associated with a higher rate

of MGUS, ranging from 3% to 29%. The severity of HIV infection is not directly associated with the presence of MGUS and does not predict progression to MM. It has been demonstrated in a few studies that when HIV is adequately controlled with cART, clinical outcomes in MM patients who undergo ASCT are no different from those of patients without HIV infection. It is important to note that zidovudine causes bone marrow suppression and may cause a delay in recovery of cell counts after ASCT. Therefore, it should be avoided as much as possible.[69,70]

A 2012 study looking at three HIV-positive patients with MM who underwent HDT-ASCT showed that all the patients achieved CR. One patient died of treatment-related complications while the other two were still in CR after four years of follow up.[69]

Another study conducted in Japan looked at outcomes in 23 HIV-positive MM patients who underwent ASCT. OS was similar between those with/without HIV infection (5-year OS 61% versus 63%).[70]

HIV in association with MM is the subject of another MMed study at our centre (which has currently been submitted for marking). The details of this and other related studies, which are well covered in the MMed research, are eagerly awaited (personal communication with the MMed student, Dr Jayson Baxter, 2023)

CHAPTER 2: PATIENTS AND METHODS

2.1. Aim

The aim of this study was to analyse the demographic and clinical profiles as well as the outcomes of adult MM patients who underwent ASCT at the Clinical Haematology Unit, Department of Medicine, CHBAH in Soweto, SA during the time period 01/01/2003 to 31/12/2021.

2.2. Objectives

Specifically, this study had the following objectives:

a) To describe the demographic profile of MM patients who underwent ASCT (e.g., age, gender, ethnic group, comorbidities).

b) To describe the clinical features and laboratory parameters at presentation and on follow-up after treatment.

c) To risk stratify patients based on the ISS (or Durie-Salmon staging where applicable), and the ISS/R-ISS staging system where feasible. A few patients were also risk stratified based on the R-ISS scoring system.

d) To describe the different induction regimens, mobilisation agents for harvesting stem cells and conditioning regimens used prior to ASCT.

e) To assess the response and complications related to ASCT.

f) To assess the response to maintenance chemotherapy post ASCT.

g) To compare the outcomes of the study when divided into 2 equal time periods (January 2003-June 2012 and July 2012-December 2021). This is relevant in order to assess the impact of novel agents in ASCT patients as they became increasingly available over the years.

2.3 Methodology

2.3.1. Study design

This study was a retrospective audit, with descriptive and comparative elements. Data was obtained from files of patients who had an ASCT at the Clinical Haematology Unit, Department of Medicine, CHBAH. The data was captured on a spreadsheet

using Microsoft Excel, an application used for data capturing and statistical analysis. Anonymity was ensured using study numbers to identify patients (See Appendix F).

2.3.2. Study population

This study included all the patients who had a confirmed diagnosis of MM and had received at least one ASCT at the Clinical Haematology Unit, Department of Medicine, CHBAH during the period 01/01/2003 to 31/12/2021 (i.e., 19 years). A sample size of 51 patients was obtained.

2.3.2.1. Inclusion criteria

- Adults ≥ 18 years old.
- Confirmed diagnosis of MM based on a combination of clinical, radiological and laboratory features including protein electrophoresis, immunofixation and SFLC assays.
- Patients who underwent ASCT during the time period January 2003 to December 2021.

2.3.2.2. Exclusion criteria

- Patients with inadequate records and clinical and laboratory information.
- Patients not fulfilling the diagnostic criteria for MM.
- Patients with MM who were not subjected to ASCT.

2.3.3. Confounding variables and limitations

Due to the retrospective nature of the study, some data was incomplete and/or missing. There was also a high rate of patients who were lost to follow-up/non-compliant, which obscured valuable information regarding longer term outcomes.

2.3.4. Data collection

Data was obtained retrospectively from patient files and captured into a spreadsheet (See Appendix A). The data was then statistically analysed using SAS Enterprise Guide 7.1. Data included patient demographics, co-morbidities, presentation

features, laboratory and other diagnostic parameters, treatment regimens, ASCT details and outcomes.

2.3.5. Sampling

A total of 51 patients were identified and were evaluable for review during the study period.

2.3.6. Ethics

Ethics approval was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, Johannesburg. Access to patients' records was obtained after getting consent from the Head of the Clinical Haematology Unit, the Head of the Department of Internal Medicine and the Chief Executive Officer of CHBAH. Patient identities were kept anonymous and raw data was only accessible to the principal investigator, supervisors and statistician. The study only commenced once permission and approval had been obtained from all the above-mentioned authorities.

2.3.7. Statistical analysis

Categorical data was analysed and presented as frequencies and percentages for the overall study and also stratified by period (January 2003 to June 2012 versus July 2012 to December 2021). To test for statistical significance for categorical measures stratified by period, Chi-Square test analysis or Fisher's test was used. Continuous measures were assessed using medians and inter-quartile ranges (IQR) as well as means and standard deviations and compared non-parametrically and parametrically using the Kruskal-Wallis test and student t-test, respectively. All statistical analysis was conducted in SAS Enterprise Guide 7.1 using the procedures FREQ, MEANS, NPAR1WAY and TTEST.

Survival time was measured in years from the date of diagnosis to the date last seen. Kaplan-Meier curves were used to compare survival outcomes, whenever feasible, between different groups.

2.3.8. Study significance

A Pubmed search showed a paucity of studies on the outcomes of ASCT in MM in the African continent. MM is a condition with increased prevalence in the African continent as life expectancy has improved in the past decade due to better access to medical care. Studies describing the patient characteristics and outcomes related to ASCT in Africa compared to European countries and USA are therefore necessary. ASCT at the Clinical Haematology Unit, CHBAH has been used since the early 2000s and analysing data regarding this treatment modality will help guide clinicians treating MM in SA and the African continent at large regarding future practices, as well as improving and optimising our local practice.

CHAPTER 3: RESULTS

3.1. Number of patients who received ASCT per year

There was a total of 691 patients diagnosed with MM over the study period (01/01/2003 to 31/12/2021). 51 of these patients (7.4 %) received ASCT(s) and were evaluable in this study.

The average number of patients newly diagnosed with MM and referred to the Clinical Haematology Unit, Department of Medicine, CHBAH per year was 36. The mean number of patients who received an ASCT per year was 2.68, with a median of three. The mean number of transplants in the second half of the study period (July 2012 to Dec 2021) was higher at 3.3 compared to the first half of the study, which was two. This reflects a heightened confidence and easier access gained over the years at the centre in offering patients ASCT.

The graph below depicts the number of ASCTs that were performed each year at CHBAH.

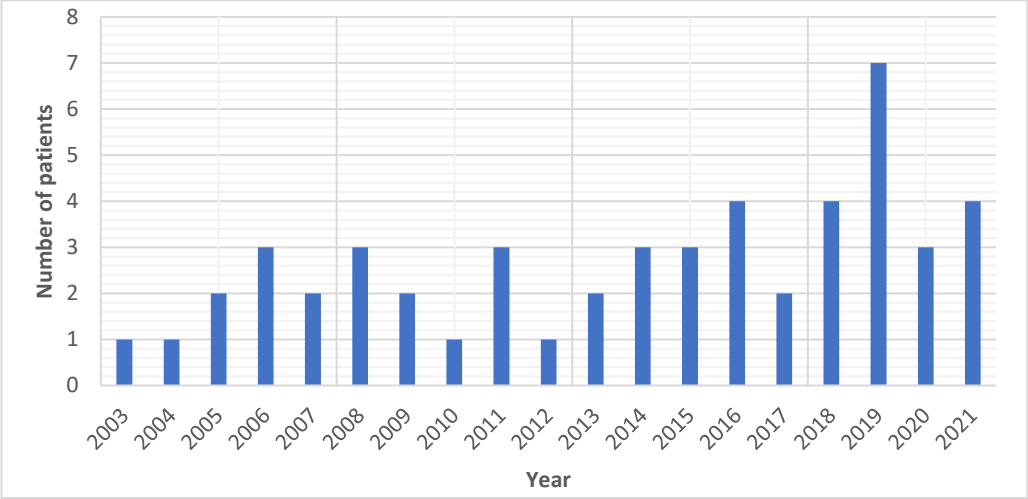


Figure 3.1: Number of patients who received autologous stem cell transplantation

3.2. Demographics of ASCT patients

There was a total of 51 evaluable patients who received an ASCT, of which 30 (58.82%) were females and 21 (41.18%) were males. The male:female ratio was

1:1.43. The median age at presentation was 54 years, with the youngest and oldest patients being 32 and 62 years of age, respectively. The different ethnicities that constituted this patient population were as follows: black patients: 86.27%, white patients: 3.92%, indian patients: 3.92% and mixed-race (coloured) patients: 5.88%. Most patients who received an ASCT had a good baseline Eastern Cooperative Oncology Group (ECOG) score, with 11.8% having an ECOG score of zero and 64.7% an ECOG score of one.

Table 3.1: Demographic characteristics of patients who received autologous stem cell transplantation

Gender	
Female (%)	30/51 (58.8)
Male (%)	21/51 (41.2)
Age (in years)	
n, Median (IQR)	51,54.0 (47.0-57)
n, Mean (SD)	51,52.0 (6.99)
n, Min, Max	51 (34-62)
ECOG score at presentation	
0 (%)	6/51 (11.8)
1 (%)	33/51 (64.7)
2 (%)	10/51 (19.6)
3 (%)	2/51 (3.9)
Ethnic group	
Black (%)	44/51 (86.3)
Indian (%)	2/51 (3.9)
Mixed race/coloured(%)	3/51 (5.9)
White (%)	2/51 (3.9)

IQR: interquartile range; SD: standard deviation; ECOG: Eastern Cooperative Oncology Group

The most common co-morbidity seen in these patients was hypertension at 64.7%, followed by diabetes at 19.6%. 7.8% of the patients had some form of renal

impairment at diagnosis which did not require acute dialysis and was reversible with conservative measures. 5.9% had concomitant well-controlled HIV. A significant proportion (19.6%) had other comorbidities (one patient with interstitial lung disease, one with upper gastrointestinal bleeding, two with vitamin B12 deficiency, one with trigeminal neuralgia, one with stable angina, one with syphilis, one with deep vein thrombosis, one with benign prostatic hyperplasia and one with obesity) that were generally unrelated to their MM status. Of note, 13.7% of these patients had no other co-morbidities.

Table 3.2: Co-morbidities in patients who received autologous stem cell transplantation

Co-morbidity	**N**	**%**
Hypertension	33	64.7
More than one comorbidity	15	29.4
Diabetes	10	19.6
Both hypertension and diabetes	10	19.6
Other	10	19.6
None	7	13.7
Renal impairment	4	7.8
HIV	3	5.9

HIV: human immunodeficiency virus

3.3. Presenting symptoms

The most common presenting symptom in this cohort of patients was bone pain (86.3%). Bone pain involving the spine afflicted 54.9% of all patients and 31.4% of patients complained of non-spinal bone pain; other significant symptoms that this group of patients presented with were, in order of decreasing frequency, fatigue (51%), weight loss (33.3%), pathological fractures and plasmacytomas (29.4% each).

Table 3.3: Presenting symptoms

Symptom		N	%
Bone pain	Total	44	86.3
	Spine	28	54.9
	Non-spine	16	31.4
Fatigue		26	51
Weight loss		17	33.3
Pathological fractures	Total	15	29.4
	Long bones/ribs	12	23.5
	Spine	3	5.9
Plasmacytoma		15	29.4
Neuropathy		11	21.6
Night sweats		6	11.8
Bleeding		2	3.9
Fever		1	2
Hyperviscosity		0	0

3.4. Clinical signs

A significant proportion of patients had bone tenderness on examination (52.9%). This is in keeping with bone involvement early in the course of MM. Pallor was also a notable finding in these patients (29.4%), although, based on the haemoglobin level, anaemia was a universal finding, occurring in 100% of the patients (see Table 3.5). Pathological fractures were seen in 29.4% of cases; 23.5% of these involved long bones and ribs and 5.9% were spinal compression fractures. Neurological findings were present in 11.8% of the patients (four patients with spinal cord compression and two with features of peripheral neuropathy).

Table 3.4: Clinical signs

Sign	N	%
Bone tenderness	27	52.9
Pallor	15	29.4
Fracture (long bones, ribs, spine)	15	29.4
Neurological	6	11.8
Thrombosis	3	5.9
Pedal oedema	2	3.9
Lymphadenopathy	2	3.9
Infection	2	3.9
Hepatomegaly	1	2
Cardiac failure	1	2
Other	1	2

3.5. Laboratory parameters

A summary of the laboratory findings is displayed in Table 3.5 below. Of note, the following improvements were seen in the median values of the following indices one year after ASCT:

- haemoglobin from a baseline of 9.65 g/dl to 12.3 g/dl.
- total serum protein level from 93 g/l to 77 g/l.
- serum albumin level from a baseline of 35 g/l to 42 g/l.
- serum paraprotein level from a baseline of 38 g/l to 9 g/l.
- serum IgG level from 33.6 g/l to 15.5 g/l.
- kappa light chain level from 68.9 g/l to 21.1 g/l; this translated to an improvement in the κ: λ ratio from 7.08 to 0.92.

The median white cell count, platelet count, creatinine, calcium, albumin and paraprotein levels at presentation were within normal ranges, notably 5.89 x 10^9/l, 268 x 10^9/l, 82 μmol/l, 2.4 mmol/l, 35 g/l and 38 g/l, respectively and remained stable up to one year after ASCT (refer to Table 3.5).

Table 3.5: Laboratory findings in patients who received autologous stem cell transplantation

	Median (Range)				
Parameter	**At presentation**	**After induction chemotherapy**	**One month post-ASCT**	**Six months post-ASCT**	**One year post-ASCT**
White cell count ($x10^9$/l)	5.82 (4.40-9.39)	5.60 (4.20-7.3)	6.12 (4.55-7.58)	5.14 (4.20-7.44)	4.84 (4.24-6.4)
Haemoglobin (g/dl)	9.65 (8.20-11.1)	12.2 (11.2-13.8)	11.8 (10.0-12.3)	12.1 (11.6-13.5)	12.3 (11.0-14.1)
Mean corpuscular volume (fl)	92.1 (86.5-95.9)	88.5 (85.0-95)	90.0 (85.0-95)	90.0 (85.0-92.6)	89.0 (86.0-94)
Platelets ($x10^9$/l)	268 (210-308)	290 (258-349)	258 (202-342)	266 (196-311)	253 (218-303)
Neutrophils ($x10^9$/l)	2.92 (2.11-4.61)	3.09 (2.21-4.29)	2.35 (1.79-2.99)	2.39 (1.81-3.71)	2.13 (1.70-2.6)
Lymphocytes ($x10^9$/l)	2.15 (1.39-2.7)	1.42 (1.04-1.9)	2.30 (1.44-3.31)	1.84 (1.37-2.35)	1.76 (1.26-2.49)
Sodium (mmol/l)	137 (134-141)	140 (138-142)	141 (137-143)	140 (138-143)	140 (138-142)
Potassium (mmol/l)	4.20 (3.80-4.4)	4.40 (3.95-4.6)	4.00 (3.60-4.5)	4.20 (3.90-4.6)	4.50 (4.20-4.7)
Urea (mmol/l)	4.40 (3.50-6.6)	3.95 (2.80-5)	3.60 (2.70-4.4)	4.55 (3.60-5.2)	4.50 (3.30-5.6)
Creatinine (µmol/l)	82.0 (64.0-105)	74.0 (62.0-84)	72.0 (62.0-85)	76.0 (63.0-87)	76.5 (59.0-91)
Calcium (mmol/l)	2.40 (2.26-2.56)	2.28 (2.21-2.4)	2.23 (2.14-2.31)	2.28 (2.19-2.37)	2.28 (2.20-2.38)
Total protein (g/l)	93.0 (75.0-106)	75.0 (68.0-79)	75.0 (69.0-78)	74.0 (70.0-78)	77.0 (72.0-80)
Albumin (g/l)	35.0 (31.0-41.5)	42.0 (38.0-44)	42.0 (39.0-43)	43.0 (39.0-46)	42.0 (40.0-44)
Paraprotein (g/l):	38.0 (24.7-61.9)	7.61 (5.00-16.4)	5.00 (3.50-10.2)	3.70 (3.00-7)	9.00 (3.00-20)
IgG (g/l)	33.6 (11.4-50.7)	13.6 (8.57-17.8)	15.2 (11.4-20.1)	13.2 (10.8-16.2)	15.5 (12.7-19.7)
IgA (g/l)	0.58 (0.40-0.91)	0.85 (0.52-1.41)	0.88 (0.58-1.46)	1.04 (0.64-1.46)	1.35 (0.92-2.27)
IgM (g/l)	0.32 (0.27-0.46)	0.42 (0.28-0.69)	0.46 (0.37-0.61)	0.58 (0.44-0.67)	0.54 (0.36-0.7)
Kappa (mg/l)	68.9 (19.0-788)	14.5 (9.80-52.1)	15.6 (8.60-29)	20.0 (11.6-26.5)	21.1 (16.1-27.5)
Lambda (mg/l)	11.8 (8.00-19.3)	12.0 (9.50-17)	16.0 (11.8-21.3)	20.4 (13.5-28.3)	22.3 (17.9-32.6)
Kappa: Lambda Ratio	7.08 (1.09-57.9)	1.30 (0.84-5.2)	0.95 (0.48-2.2)	0.78 (0.29-1.14)	0.92 (0.79-1.47)

Ig: immunoglobulin; ASCT: autologous stem cell transplantation

Table 3.6: Multiple myeloma subtypes

Subtype	**N**	**%**
IgG kappa	24	47.1
IgG lambda	10	19.6
Kappa light chain	9	17.6
Lambda light chain	5	9.8
IgA kappa	2	3.9
IgA lambda	1	2

Ig: immunoglobulin

Two patients (3.9%) had a vitamin B12 level that was low (i.e., < 145 pmol/l); 22 patients (43.1%) had an elevated beta-2 microglobulin level of more than 3.5 mg/l and 24 patients (47.1%) had an elevated baseline lactate dehydrogenase level of more than 190 U/l.

Table 3.7: Other baseline blood results at diagnosis

Iron (µmol/l)	
n, Median (IQR)	35, 10.2 (7.70-13.5)
n, Mean (SD)	35, 11.5 (5.62)
n, Min, Max	35 (3-33)
Transferrin (g/l)	
n, Median (IQR)	35, 1.98 (1.70-2.32)
n, Mean (SD)	35, 2.01 (0.47)
n, Min, Max	35 (1-3)
Transferrin saturation (%)	
n, Median (IQR)	35, 23.0 (16.0-27)
n, Mean (SD)	35, 24.4 (12.3)
n, Min, Max	35 (7-59)
Ferritin (µg/ml)	
n, Median (IQR)	36, 231 (146-434)
n, Mean (SD)	36, 385 (432)
n, Min, Max	36 (26-1634)
Vitamin B12 (pmol/l)	
n, Median (IQR)	35, 344 (247-655)
n, Mean (SD)	35, 454 (282)
n, Min, Max	35 (95-1280)
Serum folate (nmol/l)	
n, Median (IQR)	13, 12.9 (11.1-23.4)
n, Mean (SD)	13, 17.4 (9.80)
n, Min, Max	13 (6-39)
Uric Acid (mmol/l)	
n, Median (IQR)	33, 0.36 (0.25-0.43)
n, Mean (SD)	33, 0.36 (0.15)
n, Min, Max	33 (0-1)
Beta-2 Microglobulin (mg/l)	
n, Median (IQR)	34, 4.90 (2.70-8.6)
n, Mean (SD)	34, 6.29 (5.47)
n, Min, Max	34 (1-30)
Lactate Dehydrogenase (U/l)	
n, Median	33, 256
n, Mean	33, 300
n, Min, Max	33 (111-990)

IQR: interquartile range; SD: standard deviation

3.6. Imaging studies

The most common bone lesions seen on initial imaging studies (radiographic skeletal survey, CT scan, MRI scan) were lytic lesions (70.2%), followed by fractures in 48.9% of the patients. Vertebral compression fractures were picked up in 16 patients (31.4%) on MRI scan and 12 patients (23.5%) presented with long bone/rib

pathological fractures on initial imaging. Other non-specific and age-related osteodegenerative lesions were picked up in 34% of patients. A number of patients (54.9%) had more than one bone lesion (skeletal-related events).

The findings of the skeletal survey were available for 33 patients, out of whom lytic lesions were seen in 20 (60.6%), fractures in five (15.2%) and a combination of lytic lesions and fractures in eight patients (24.2%), respectively. Out of the 10 patients who had CT scans, four (40%) had at least one fracture, two (20%) had lytic lesions and four (40%) had bone or soft tissue masses suggestive of a plasmacytoma. A spinal MRI scan was done in 23 patients, with four (17.4%) showing osteodegenerative changes and three (13%) showing a soft tissue mass, in addition to the 16 patients (69.6%) showing vertebral compression fractures, as described above.

Table 3.8: Imaging findings in patients who underwent autologous stem cell transplantation

Bone lesion	N	%
More than 1 bone lesion	28	54.9
Osteolytic lesions	8	15.7
Fractures	7	13.7
Missing data	4	7.8
Other lesions	3	5.9
Plasmacytomas	1	2

3.7. Bone marrow analysis

3.7.1. Clonal plasma cells

Information on bone marrow plasmacytosis was available for 46 patients. Of these patients, 69.6% had clonal plasma cells of at least 10% on either the bone marrow aspirate or trephine; 17.4% of the patients displayed plasmacytosis of ≥ 60% in keeping with a biomarker of malignancy and 13% displayed a plasmacytosis of less than 10% (however, there were enough other criteria to establish the diagnosis of MM in these patients).

3.7.2. Flow cytometry

Flow cytometry results were available for 32 of the patients in the study. All of them showed positivity for CD38, CD138 and CD56, which is in keeping with a finding of abnormal or aberrant plasma cells. Out of the 30 immunophenotype panels that were reported for κ and λ light chains, 10 of them (33.3%) showed a kappa light chain restriction and five (16.7%) showed lambda light chain restriction; 15 (50%) showed a normal κ: λ ratio.

Table 3.9: Immunophenotype in patients who had autologous stem cell transplantation

Immunophenotype	
CD38	
n, Median (IQR)	32, 80.5 (59.0-90)
n, Mean (SD)	32, 70.0 (28.4)
n, Min, Max	32 (3-98)
CD138	
n, Median (IQR)	32, 5.25 (2.60-16.1)
n, Mean (SD)	32, 13.7 (19.5)
n, Min, Max	32 (0-83)
CD56	
n, Median (IQR)	32, 23.5 (11.8-41.8)
n, Mean (SD)	32, 29.1 (22.7)
n, Min, Max	32 (1-88)
CD20	
n, Median (IQR)	15, 2.00 (0.80-4.5)
n, Mean (SD)	15, 8.52 (22.1)
n, Min, Max	15 (0-88)
Kappa	
n, Median (IQR)	30, 2.05 (1.00-20)
n, Mean (SD)	30, 12.9 (20.7)
n, Min, Max	30 (0-68)
Lambda	
n, Median (IQR)	30, 0.80 (0.50-2.6)
n, Mean (SD)	30, 9.07 (21.3)
n, Min, Max	30 (0-80)

CD: cluster of differentiation; IQR: interquartile range; SD: standard deviation

3.7.3. Cytogenetics

Of the 15 patients who had cytogenetics done on their bone marrow samples, 80% demonstrated a low-/intermediate-risk profile, while 20% had high-risk features [one had t(4;14), one had t(14;20) and one had del 17p]. Of the patients with low-

/intermediate-risk cytogenetics, four were reported as being hyperdiploid, one as t(11;14) and two as del 13q.

Table 3.10: Cytogenetics in patients who received autologous stem cell transplantation

Cytogenetics profile	N	%
Low-/intermediate-risk	12	80
High-risk	3	20
Data missing	36	-

3.8. Staging

Forty-one patients had data available to classify them as per the ISS score; 32 % were ISS I, 32 % ISS II and 36 % were ISS III. In terms of the Durie Salmon staging, data was available for 50 patients, which put the majority (86%) into class IIIA.

Table 3.11: International Staging System staging

Stage	N	%
III	15	36
I	13	32
II	13	32

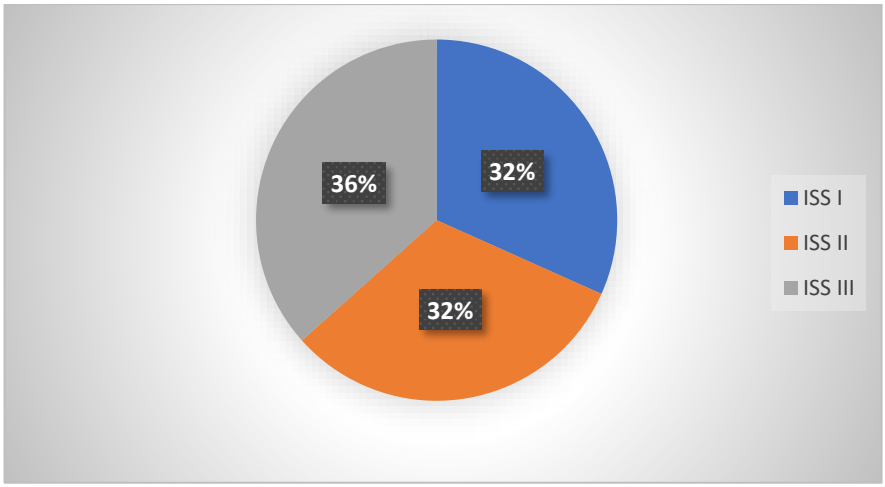


Figure 3.2: International Staging System (ISS) staging

Table 3.12: Durie Salmon staging

Stage	N	%
IIIA	43	86
IIIB	4	8
IA	2	4
IIA	1	2

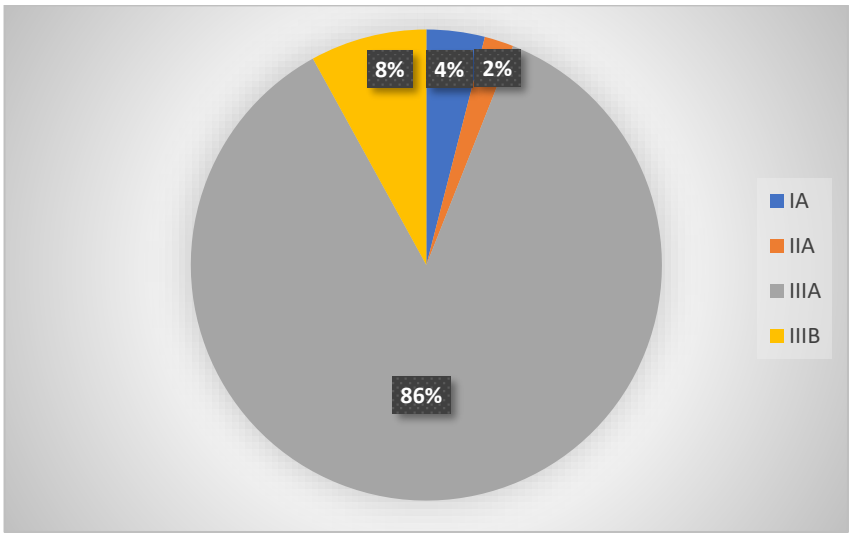


Figure 3.3: Durie-Salmon Staging

3.9. Induction chemotherapy

Data regarding the induction chemotherapy regimen for patients before the first ASCT was available for 47 patients. Information was lacking from two files and the induction chemotherapy regimens were not clearly documented in the remaining two files. The mean number of induction chemotherapy cycles required to achieve at least a partial response (PR) before a patient was considered for ASCT was 9.5 cycles for the entire cohort. The median was eight cycles. Most of these induction regimens consisted of modified CVAD (85.1%), one patient was treated with VTD, one with melphalan and prednisone and five patients (10.6%) required more than one induction regimen before ASCT was performed (one patient received CTD in private, then VTD at our facility, four patients received CVAD followed by CTD).

The median time between diagnosis of MM and receiving the first ASCT was 17 months (range 6-72 months), with a mean of 23.2 months for the entire cohort.

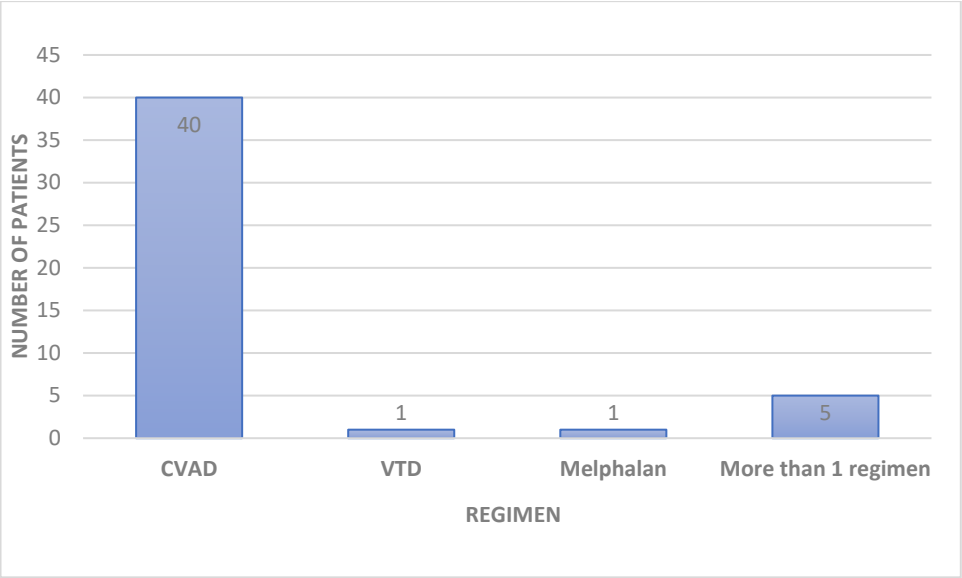


Figure 3.4: Induction chemotherapy before the first autologous stem cell transplantation

3.10. Stem cell harvest

All patients in the study received a single dose of intravenous etoposide 400 mg as the mobilisation agent for stem cell harvest. It was administered approximately 12 days before the harvest and one week before G-CSF (filgrastim 5µg/kg twice daily for five days) was given.

The median $CD34^+$ yield obtained for ASCT was 9.83 x 10^6/kg (range 2-36 x 10^6/kg) out of the 47 patients on whom this data was available. This was sufficient for at least a single ASCT for all these patients. Seventeen (36.2%) patients underwent a single apheresis session, 21 (44.7%) had 2 apheresis sessions and nine (19.1%) had three sessions to obtain the desired yield.

3.11. High-dose therapy/ASCT

3.11.1. Conditioning regimen

Melphalan was used as the preparative regimen in all the patients in the study. Most of the patients (80%) received a total dose of 200 mg/m^2 of melphalan, usually administered as 100-mg/m^2 intravenous infusions over 30-60 minutes for two consecutive days.

Table 3.13: Doses of melphalan used

Dose of melphalan	N	%
200 mg/m^2	41	80.4
Total dose of 250 – 350 mg	9	17.6
Total dose of 170 mg	1	2

Neutrophil engraftment (number of days for the absolute neutrophil count to improve to greater than 0.5 x 10^9/l without growth factor support) after high-dose melphalan occurred after a median of 11 days, with a range of 3-14 days. Platelet engraftment (number of days for the platelet count to improve to greater than 20 x 10^9/l without any platelet transfusion support) took place after a median of 10 days, with a range of 6-15 days.

3.11.2. ASCT

Fourty-one patients received a single ASCT. The 10 patients who received a second ASCT had done so as a salvage strategy following relapse after the first ASCT. 25.5% of the patients underwent an early ASCT ≤ 12 months from the date they were diagnosed with MM. 74.5 % had a delayed ASCT.

Table 3.14: Characteristics of autologous stem cell transplantation

	Number of patients (N)	%
Type of ASCT		
• Single	41	80.4
• Double	10	19.6
Timing of ASCT		
• Delayed	38	74.5
• Early	13	25.5

ASCT: autologous stem cell transplantation; Early ASCT: ≤12 months from date of diagnosis

3.11.3. Complications post-HDT/ASCT

Information regarding complications arising from HDT was available for all the 51 patients. Early complications from high-dose therapy encountered within the first two weeks were mostly related to myelosuppression, which is a well-recognised

complication of high-dose melphalan. 90.2 % of the patients developed grade IV neutropenia followed by 82.4 % of patients who developed different degrees of thrombocytopenia. Fourteen (33.3%) and 23 (54.8%) patients developed WHO grades III and IV thrombocytopenia, respectively. Anaemia was seen in 52.9% of patients. GIT complications were also quite commonly seen at 39.2%, with mucositis contributing to a large proportion of these patients. Of the five patients who developed infections, two had pneumonias, one had reactivation of herpes zoster, one developed clostridium difficile colitis and the source of infection in the remaining patient was not specified.

Table 3.15: Early complications of high-dose therapy

Complication	**N**	**%**
Neutropenia (absolute neutrophil count < 0.5 x 10^9/l)	46	90.2
Thrombocytopenia (Platelets < 100 x 10^9/l)	42	82.4
Anaemia (Haemoglobin < 10 g/dl)	27	52.9
Gastrointestinal tract (mucositis, nausea, vomiting, diarrhoea)	20	39.2
Infections	5	9.8

Late complications were seen two weeks after receiving HDT and resulted mostly from the myelosuppressive effects of melphalan, albeit at a reduced frequency. Nine patients complained of alopecia. The two patients who developed infections did not have the source of the infection reported in their files.

Table 3.16: Late complications of high-dose therapy

Complication	N	%
Neutropenia	23	45.1
Anaemia	15	29.4
Thrombocytopenia	12	23.5
Alopecia	9	17.6
GIT	6	11.8
Infection	2	3.9
Bleeding	1	2

3.12. Maintenance chemotherapy

Thalidomide monotherapy was given to 29 patients after their ASCT. Significantly more patients were on thalidomide in the second half (26 versus 3) due to its increased availability in the latter part of the study.

Melphalan, which used to be the older maintenance chemotherapy agent, was given to seven patients in the first half of the study. Lenalidomide monotherapy was given to only one patient in the latter half of the study. Six patients did not receive any maintenance agent: four of them demised before any maintenance therapy could be started and the remaining two patients were lost to follow up shortly after ASCT. Information regarding maintenance chemotherapy was not clear from one patient's file.

Five patients received thalidomide and melphalan in succession, one patient received thalidomide followed by lenalidomide and one patient received thalidomide, melphalan and lenalidomide at different times.

Thalidomide was given as maintenance treatment for a median of 32.5 months (range 5-103 months), melphalan for a median of 11 months (range 5-53 months) and lenalidomide for a median of 18.5 months (range 10-27 months).

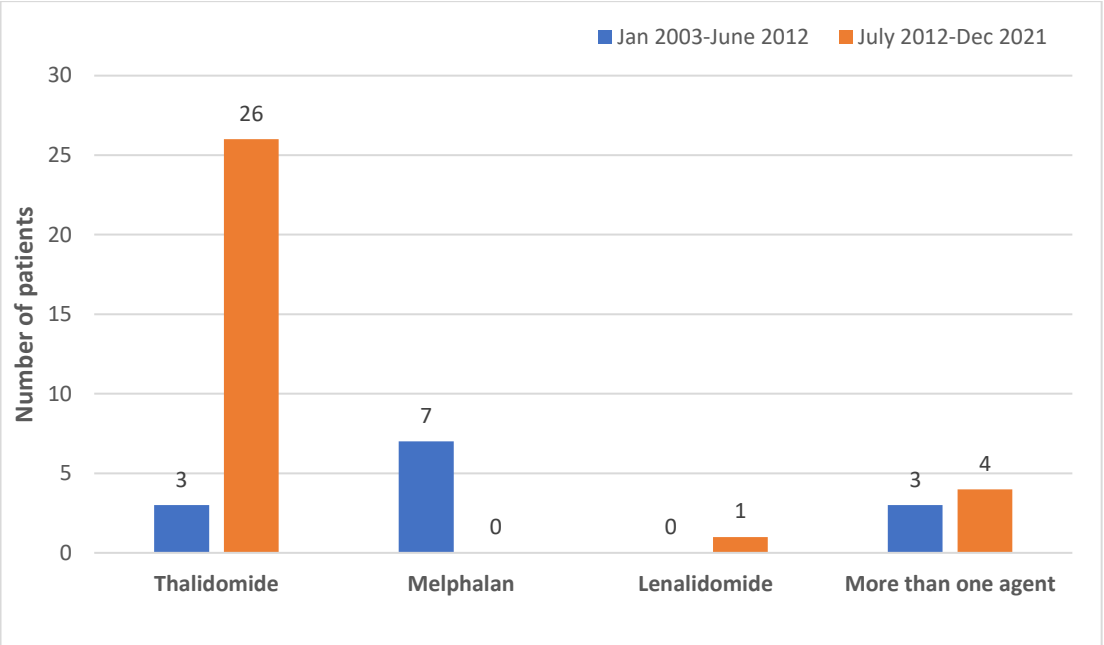


Figure 3.5: Maintenance chemotherapy after autologous stem cell transplantation

3.13. Supportive therapy

Several supportive measures were offered to these patients along with specific therapy. All the patients received antibiotics at some point in time either empirically or to treat an active infection during their illness. A high proportion of patients also required bisphosphonates in the outpatient setting to treat their myeloma bone disease. Radiotherapy was used in 41.2% of patients to treat plasmacytomas and spinal cord compression. The same percentage of patients also required transfusion of blood products at some point in their disease course, mostly to improve blood counts, post HDT.

Table 3.17: Supportive measures

Treatment	N	%
Antibiotics	51	100
Analgesia	49	96.1
Allopurinol	49	96.1
Bisphosphonates	46	90.2
Radiotherapy	21	41.2
Blood products	21	41.2

3.14. Outcomes

The outcomes of the patients in our study were based on the status of their disease at the last visit to the Clinical Haematology unit. The median OS in our patient population was 57 months from the time of diagnosis, with a range of 12 to 209 months.

Twenty-nine patients (56.9%) had demised or presumed to be dead as per their last visit notes. All the patients from the first half of the study and 33.3% from the latter half of the study had demised. Of all the patients who demised, 18 (62.1%) were showing disease relapse. Of the eight patients who did not relapse and had died, four had sepsis, one had fulminant heart failure, one died from complications related to multiple other comorbidities and two died of unknown causes.

In terms of the ISS stage, the median OS for patients in stages I, II and III were 55 months, 82 months and 55 months, respectively.

Out of the 22 patients who were alive at the conclusion of the study, two (9.1%), five (22.7%) and 10 (45.5%) were in PR, VGPR and CR, respectively. Four (18.2%) had disease relapse.

The disease response status of four patients (7,8%), three of whom died and one is alive, after ASCT was not clearly defined or not available from their respective files.

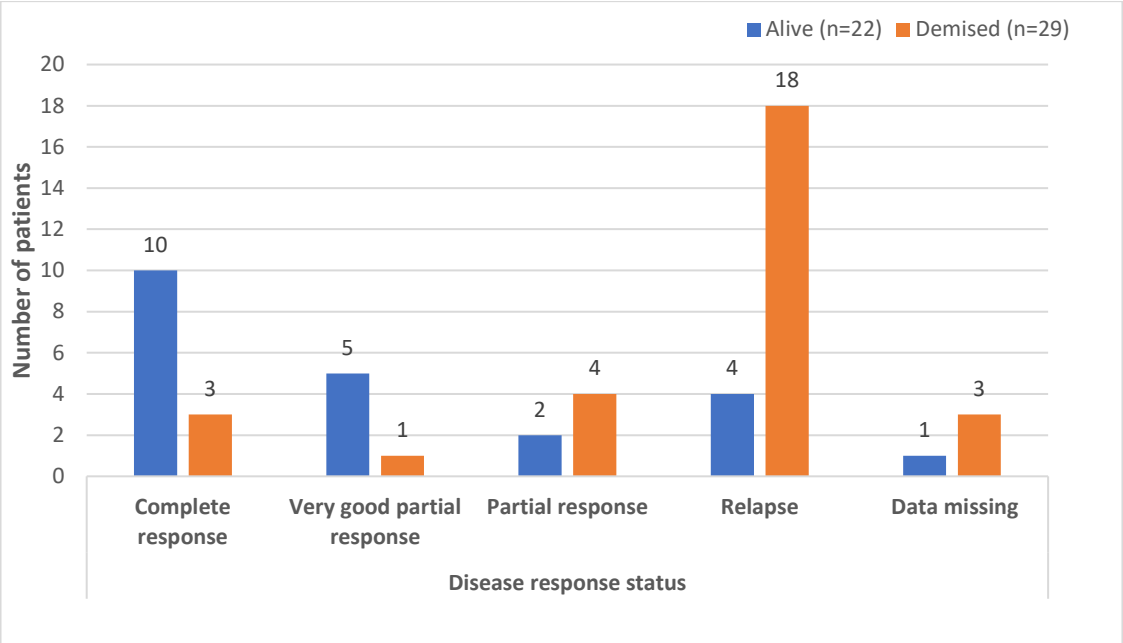


Figure 3.6: Survival and disease response status at the conclusion of the study

When the response status six months after the first ASCT was analysed for every patient, the following findings were noted:

- 19 patients had a favourable response to ASCT: three patients improved from PR to CR, 11 patients improved from PR to VGPR and five patients improved from VGPR to CR status
- 27 patients stayed in their pre-ASCT response status: 13 in PR, seven in VGPR and seven in CR
- One patient relapsed from a pre-ASCT PR status

Four patients' response status could not be interpreted at six months as three had demised prior to that and one patient defaulted further follow up appointments soon after his ASCT.

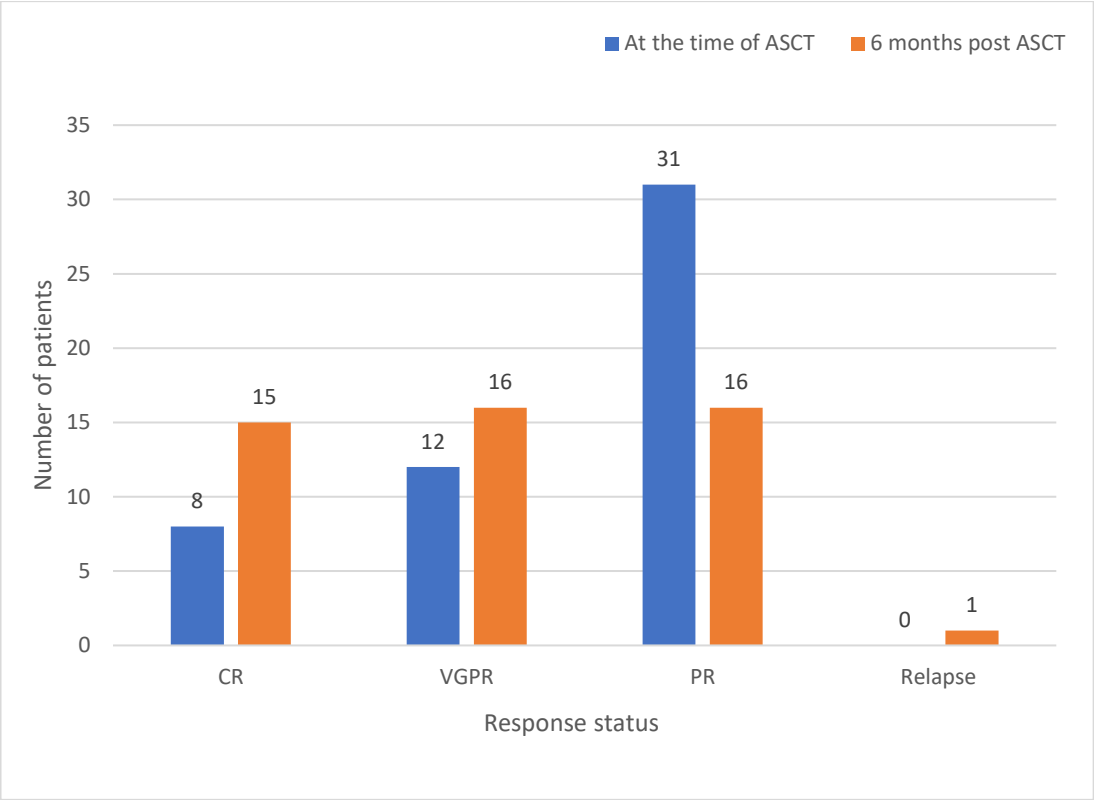


Figure 3.7: Response status at the time of the autologous stem cell transplantation and 6 months post-autologous stem cell transplantation

The median OS in the early ASCT group was 32 months compared to 58.5 months for the delayed ASCT group. 69.2 % of the patients in the early ASCT group had demised at the last visit compared to 52.6 % of patients in the delayed group. In terms of disease response status, 54.5% and 55.5% were in PR or better status in the early and delayed ASCT groups, respectively.

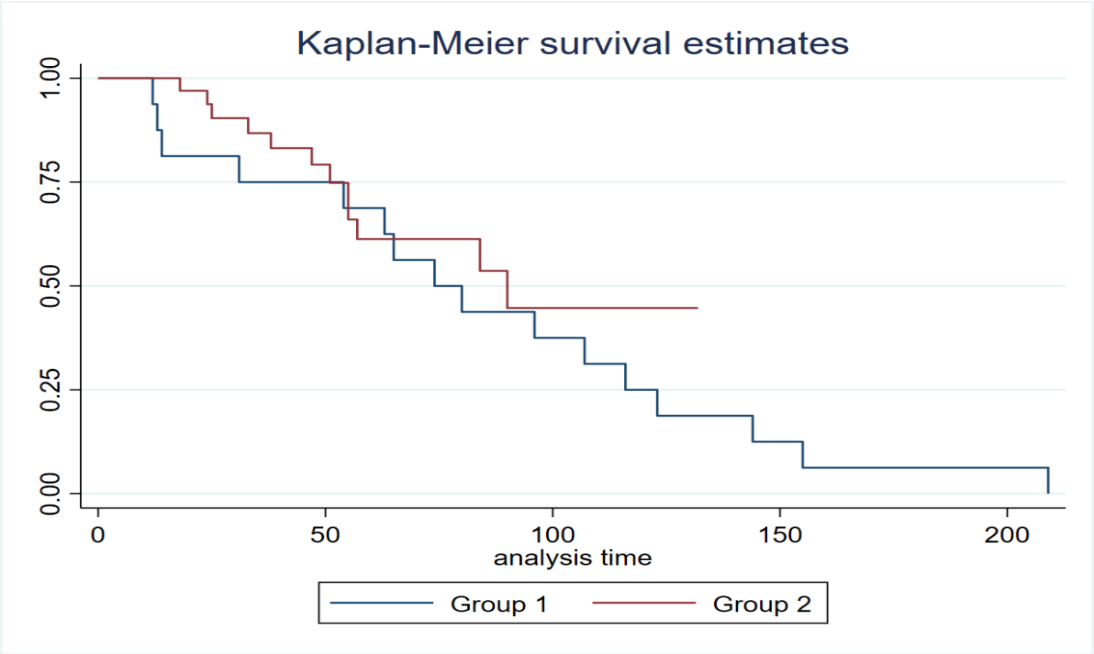
Table 3.18: Comparison of outcomes in early versus delayed autologous stem cell transplantation

Parameter	Early ASCT(n=13)	Delayed ASCT (n=38)
Median overall survival (months)	32	58.5
Response status, n (%)	**n=11**	**n=36**
• Relapse/Progressive disease	5 (38.5)	16 (42.1)
• CR	4 (30.7)	9 (23.7)
• Data unavailable	2 (15.4)	2 (5.3)
• VGPR	1 (7.7)	6 (15.8)
• PR	1 (7.7)	5 (13.1)
Survival status, n (%)	**n=13**	**n=38**
• Dead	9 (69.2)	20 (52.6)
• Alive	4 (30.8)	18 (47.4)

ASCT: autologous stem cell transplantation CR: complete response, PR: partial response, VGPR: very good partial response

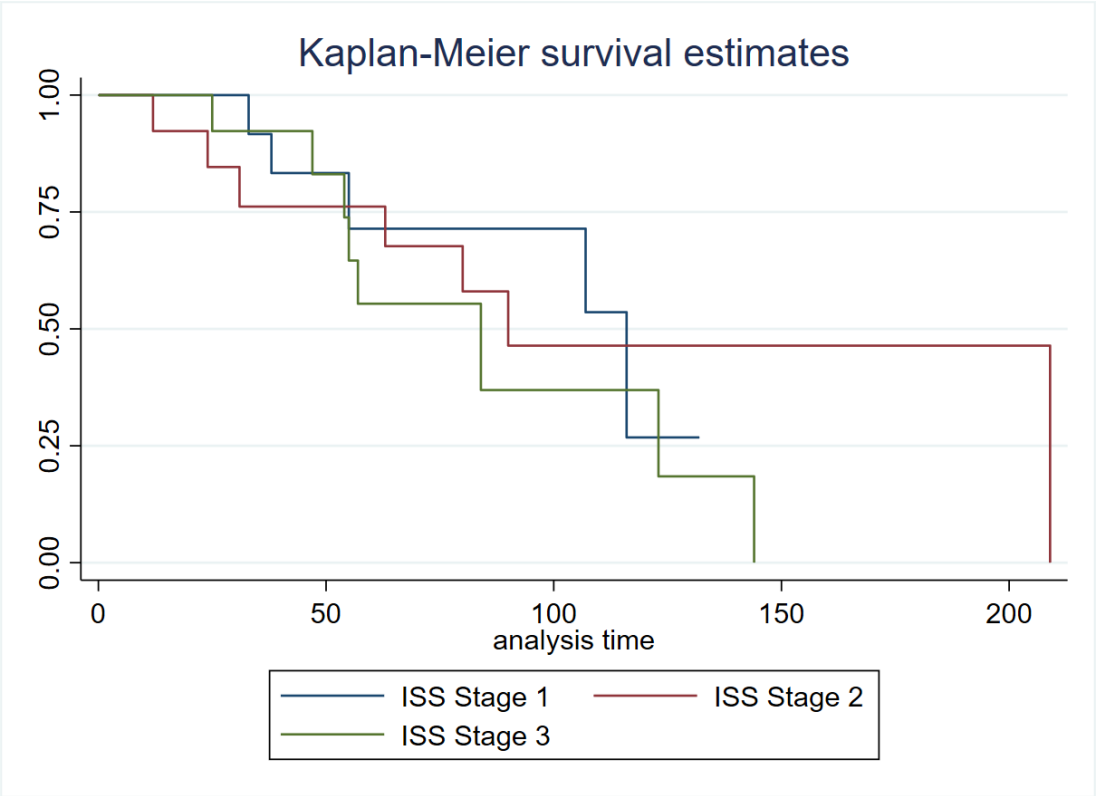
Survival data were compared between the two halves of the study. As mentioned earlier, all the patients in the first half of the study had demised at the time of analysis as opposed to only a third of the patients in the second half. A five-year predicted survival analysis for the second half of the study could not be done due to the small number of patients. Survival between the two groups was statistically not significant (log rank test p value = 0.45).

Furthermore, no statistical significance was noted when survival trends were compared between the different ISS staging groups (Figure 3.9) and between the early and delayed ASCT groups (Figure 3.10), with log-rank test p values of 0.71 and 0.12, respectively.



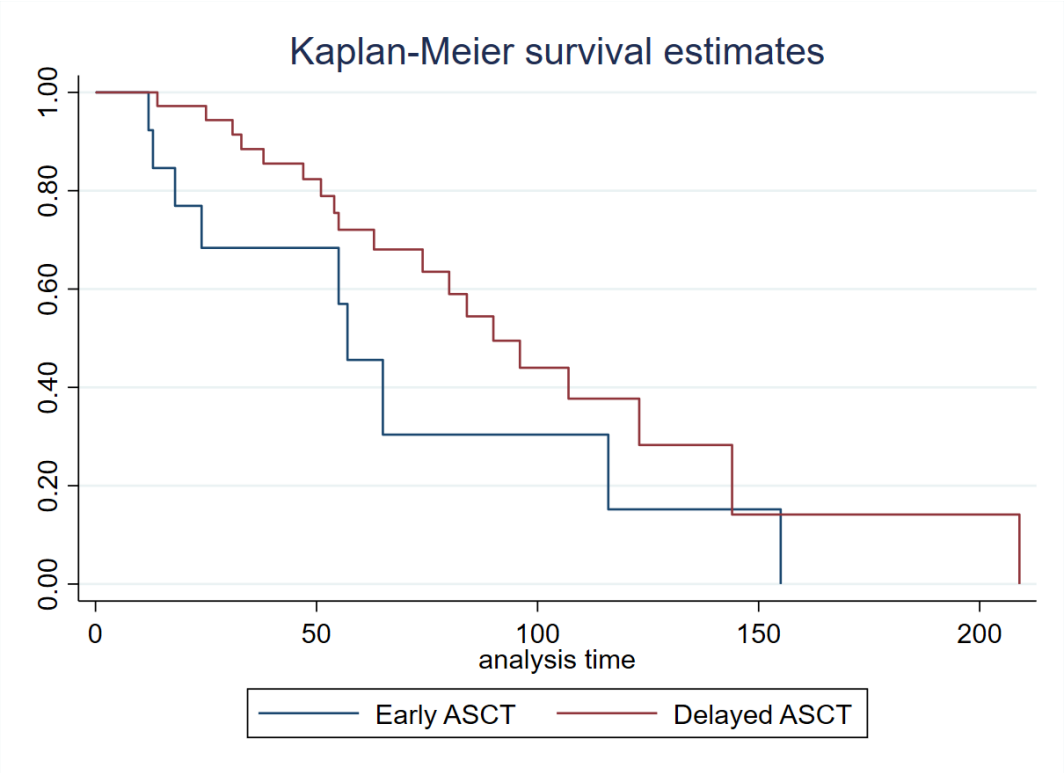
Group1: January 2003-June 2012; Group 2: July 2012-December 2021; analysis time in months

Figure 3.8: Survival in the two halves of the study



ISS: International Staging System; analysis time in months

Figure 3.9: Survival based on International Staging System groups



ASCT: autologous stem cell transplantation; analysis time in months

Figure 3.10: Survival in early versus delayed autologous stem cell transplantation groups

3.14.1. Relapse

Twenty-three patients relapsed at some point after ASCT in this study. The median time for relapse after the first ASCT was 30 months. 69.6% of the patients relapsed after the first ASCT. 30.4 % of the relapsed patients had done so after their second ASCT.

Table 3.19: Characteristics of patients who relapsed

Relapse (n=23)	
After first ASCT, n (%)	16 (69.6)
After salvage ASCT, n (%)	7 (30.4)

ASCT: autologous stem cell transplantation

CHAPTER 4: DISCUSSION

Multiple myeloma is a haematological malignancy that results from unchecked proliferation of a clone of antibody-producing plasma cells. The disease results in a number of MDE, among which the most common are bone disease, hypercalcaemia, renal involvement and anaemia. The disease is unfortunately incurable.

This study looked at the patient characteristics and outcomes associated with the practice of ASCT in the management of MM patients at a South African haematology centre. ASCT has been shown in several trials to be associated with improved PFS and OS, both before and after the introduction of the novel induction agents.[37,40,41]

4.1. Demographic findings

Out of a total number of 691 patients with MM who were seen at our facility from 2003 to 2021, 51 (7.4%) received an ASCT. The mean number of transplants per year was 2.68. This contrasts with the number of ASCT offered in Europe and the USA, where this treatment modality is a much more common practice and is offered to most patients eligible for it.[48] The male:female ratio in our cohort of patients was 1:1.4. This closely correlates with the male:female ratio of 1:1.6 in transplanted MM patients seen in the Cape Town study by Novitzky el al., 2010.[71] The cut-off age to offer ASCT in major clinical trials around the world has commonly been ≤ 65 years as these individuals are able to better tolerate HDT and its associated side effects, have fewer comorbidities and a better performance status as compared to their older counterparts.[63,67] The median age of our transplanted patients was 54 years, which was in line with the international recommendations. The majority of the patients in our review were of black ethnicity (86.3%), which forms the major ethnic group in the population of patients seen at our hospital. The most commonly associated co-morbidity was hypertension (64.7%) followed by diabetes mellitus (19.6%). Three (5.9%) of the patients had HIV and they all had a suppressed viral load. The two most common symptoms expressed by our patient cohort were bone pain (86.3%) and fatigue related to anaemia (51%). These findings correlate with the two previous

studies done at the same centre.[4,5] The most common clinical signs were bone tenderness (52.9%), pallor and pathological fractures (29.4% each). IgG myeloma was the predominant subtype (66.7%) in this group of patients, followed by light chain myeloma (27.4%). These findings are not too different from those of another study from Cape Town, SA, where IgG MM was found in 52% and light chain MM in 26% of patients who underwent ASCT.[71]

Anaemia (haemoglobin concentration of ≤ 12 g/dl) was seen in 83.3% of our patients at presentation; the earlier review done at our centre showed anaemia in 82.4% of newly diagnosed MM patients.[4] The baseline median values of the serum albumin, creatinine, beta2 microblobulin and LDH were 35 g/l, 82 µmol/l, 4.9 mg/l and 259 U/l, respectively.

Osteolytic lesions were the most common radiological finding, seen in 70.2% of the patients. Pathological fractures were present in 48.9%. This contrasts significantly with the findings of the earlier study done at our centre, where 14.8 % of the patients had pathological fractures on presentation.[4] This can be explained by the fact that our patient cohort lived longer in general due to the treatment modalities they received and were more likely to develop bone disease at some point due to disease progression. Another reason for this might be the selection of patients with more severe disease, including bone disease, as candidates for ASCT. A more recent study done in Kwazulu-Natal, SA, found that 47% of newly diagnosed myeloma patients presented with pathological fractures. This abnormally high incidence of fractures was explained by the late presentation and diagnosis of this cohort of patients. Most patients in that review presented more than six months after their symptom onset due to a lack of access to medical facilities.[72]

4.2. Induction chemotherapy

The new standard of care in terms of induction treatment is a triplet combination including and IMiD, a PI and dexamethasone.[26] In our study, most of the patients received a VAD-based regimen (85.1%), which used to be the standard induction

approach for about 30 years before the introduction of the novel agents. One patient with high-risk cytogenetics was treated with VTD. Melphalan was given to a patient who initially had paraplegia and was deemed not a good candidate for ASCT initially, but was later subjected to an ASCT after he recovered from the paraplegia. Five patients (10.6%) received more than one induction regimen before an ASCT. Induction therapy with novel agents like thalidomide and bortezomib have been shown to be associated with better survival outcomes in a number of trials.[9,38,41] At our institution, these agents have been used scarcely as induction agents, mostly due to procurement difficulties and access to these agents owing to their expensive cost. CHBAH is a public sector institution that renders medical care to patients who generally do not have access to medical insurance. At the time of writing this report, bortezomib was still largely unavailable across the public sector in SA. In the state sector, thalidomide is accessed mostly as a buy-out from private pharmacies and was therefore limited for use as maintenance chemotherapy in the past. Its routine use for induction chemotherapy has therefore not been a common practice at out treatment facility. Only one of the patients from the study received a novel agent-based induction regimen in the first half of the study. This reflects the difficulty in acquiring these agents in the South African state sector during the study period, as opposed to their regular used in the developed world.

With novel agents, the number of induction cycles recommended to achieve an acceptable level of response before considering ASCT is 3-4. Another reason is to limit the amount of exposure of the bone marrow to the myelosuppressive effects of lenalidomide.[16,35] In this study, the median number of cycles of induction chemotherapy was eight. The reasons for requiring a longer induction period were likely due to the inferior effectiveness of the older VAD regimen to achieve at least a PR response status as compared to novel drugs. Secondly, some patients received more than one induction regimen, as and when more suitable induction drugs became available later, which resulted in prolonged periods of induction chemotherapy in order to achieve the desired initial response prior to an ASCT.

4.3. Stem cell harvest

Cyclophosphamide (3-4 g/m^2) alone or combined with etoposide, yields significantly more stem cells than growth factor–only mobilisation.[53] In our setting, the mobilisation agent used in all patients was etoposide. This was combined with G-CSF (e.g., filgrastim) to improve the yield at apheresis. The use of cyclophosphamide was not common practice in our study. This is likely due to prior exposure to cyclophosphamide in the induction regimens used at our transplant facility.

The minimum number of stem cells that is required for successful engraftment at ASCT is 2 x 10^6 CD34+ cells/kg for a single transplant and ideally a total dose of 4-5 x 10^6 CD34+ cells/kg, split into two equal aliquots, in anticipation of a tandem/salvage ASCT.[53] This was the case in our setting as well, with a median number of 9.83 x 10^6 CD34+ cells/kg having been obtained. This was split into two aliquots and was sufficient to cover for a potential second ASCT for most patients in the study.

4.4. Eligibility for ASCT

The age cut-off for patients being eligible for ASCT is generally < 65 years and less in most clinical trials. This age has slowly increased to include patients up to 75 years old with a good functional status in some American studies.[48,51] The median age at diagnosis in our patient population was 54 years. The oldest patient in our study who received a transplant was 62 years, which was in line with the standard for ASCT at the time. Even in the second half of our study, there were no patients > 65 years who were considered for an ASCT. There were many plausible reasons for this. Firstly, most of the patients included in our study had one or more comorbidities. Only 13.7% of the patients in our study had no co-morbidities. Secondly, most of the patients who received an ASCT came from a disadvantaged socioeconomic background and some of them had to travel from far, sometimes from other provinces, to come for their treatment and follow up appointments. This required a younger, fit patient who would be able to come for regular visits to the hospital and tolerate the high doses of chemotherapy drugs with their associated side effects.

Forty-nine of our patients had a good ECOG PS score of ≤ 2 at baseline. Four patients in the study had baseline renal impairment which subsequently improved before they were considered for an ASCT. This was in line with the eligibility criteria for ASCT, especially for elderly fit patients who would be able to tolerate lower doses of melphalan in the setting of renal impairment.[55]

4.5. Conditioning regimen

All the patients in this study were in line with international guidelines in terms of melphalan being used as an effective conditioning drug for high-dose therapy. Forty-one patients received the usual 200 mg/m^2 of the drug. The other 10 patients received varied doses based on their respective weights, with nine patients requiring more than 200 mg/m^2 in total. None of our patients had ongoing renal dysfunction at the time they received melphalan.

The increase in the amount of reinfused stem cells reduces the risk of complications and hospitalization by shortening the neutrophil and platelet engraftment times.[9] In our setting, the amount of stem cells reinfused was > 2 x 10^6 CD34+ cells/kg as most patients had an adequate amount of stem cells collected to cater for at least two ASCT and the common practice at our institution was to split the collected stem cells into two aliquots. The median engraftment times for ANC and platelets were 11 and 10 days, respectively, which was comparable to the study done by Bashir et al., 2019, where they also used melphalan 200 mg/m^2 as the conditioning regimen.[56] The Cape Town study by Novitzky et al., 2010, showed slightly longer engraftment times of 13 and 17 days for ANC and platelets, respectively.[71]

4.6. Early versus delayed ASCT

Early ASCT compared to a delayed strategy has been shown to have better PFS but not OS.[40,57] The median OS in our patient cohort who received an early ASCT within 12 months of diagnosis was 32 months compared to 58.5 months for patients who received a delayed ASCT. This was non-significant (p=0.12) and confirms the fact

that a delayed approach was safe and effective in our patient population, having not impacted adversely in relation to the OS.

4.7. Single versus Tandem ASCT

Tandem ASCT has been shown to be particularly helpful for those patients with high-risk cytogenetics.[47] In our patient population, most patients underwent a single ASCT and it was generally not the practice to perform tandem ASCT. There were a number of reasons for delays in receiving an ASCT in our patient cohort. Some patients, after counselling, were uncertain and unwilling to commit to the procedure despite agreeing to it and having had a stem cell harvest. High dose chemotherapy agents and growth factors were not always readily available and the ASCT procedure had to be timed according to the availability of these drugs. Additionally, at times, there were unplanned delays on the part of the Clinical Haematology Unit, based on the availability of the clinical and nursing staff.

4.8. Salvage ASCT

Most patients with MM experience a relapse at some point, despite consolidation and/or maintenance therapy after ASCT. Several retrospective studies have evaluated the role of salvage ASCT in the relapse setting, and they have demonstrated that ASCT for a second or even third time is a feasible and effective treatment option among patients who previously underwent ASCT.[64,65] In our setting, 10 patients were subjected to a salvage ASCT after relapse. Of those 10 patients, two displayed high-risk cytogenetics on their marrow sample [one patient had del 17p and one had t(4;14)] and two displayed standard-risk cytogenetics. Four of those patients had ISS stage III disease and another patient had Durie-Salmon stage IIIA disease at baseline.

4.9. Complications related to ASCT

The most common complications related to high-dose therapy within the first two weeks were, as expected, related to the myelosuppressive effects of high-dose melphalan. A significant proportion of the patients developed neutropenia and they were covered empirically with antibiotics, which is the standard practice at our

centre. Many patients also developed thrombocytopenia and anaemia requiring transfusion of packed cells or platelets. Stomatitis, including mucositis and diarrhoea were other common complications in the immediate HDT/ASCT setting.

Three patients died of treatment-related complications, with two of them succumbing to sepsis within the same admission and another patient dying of a pneumonia three months after his ASCT. He was in PR at the time of his death.

The low incidence of infection was likely because empiric antibiotics were prescribed pre-emptively and most of these patients were treated in an isolation room, with the appropriate neutropenic measures.

The late complications were mostly attributable to the residual myelosuppressive effects of HDT.

4.10. Maintenance chemotherapy

Post-transplantation maintenance therapy currently involves the use of 1-2 novel agents at a lower dose than that used for induction with the goal of maintaining the depth of response achieved by ASCT or achieving MRD negativity. In our setting, 36 patients (70.6%) received thalidomide as their maintenance chemotherapy agent. Melphalan was the predominant chemotherapy agent used early in the study when thalidomide was still not accessible in South Africa. Lenalidomide is still not an easily accessible drug in the public sector hospitals in South Africa with only two patients having benefited from its use in this review.

The most common side effects reported by patients were peripheral neuropathy (eight patients) and constipation (12 patients). They were thus on empiric laxatives and, in patients with neuropathy or neutropenia, the dose was reduced or stopped as per the severity of the complication. Two patients had developed deep venous thrombosis while on thalidomide, despite our patients being routinely put on thromboprophylaxis

with aspirin (75 mg daily). The dose of thalidomide used for maintenance therapy was typically 50-100 mg daily and generally did not exceed 200 mg daily.

4.11. Outcomes

Overall survival in the era before ASCT was usually less than 18 months with conventional chemotherapy.[13,26] The median OS in our cohort of patients was 57 months (range: 12-209 months). An earlier study done at our centre and which looked at newly diagnosed MM patients showed an OS of 28.4 months.[4] Another review study done at our centre showed that the survival rate beyond 12 months among newly diagnosed MM patients was only 35.2%.[5] Both these studies, however, looked at survival outcomes in all newly diagnosed MM patients irrespective of whether they received treatment or not. This shows the clear benefit that ASCT had in prolonging our patients' life expectancies.

There was no statistically significant impact of ASCT when the OS between the two halves of the study was compared ($p=0.45$). However, the predicted OS over a longer time-period in the latter half of the study could not be calculated due to the small number of patients. This might have been a useful piece of information to assess the impact on survival of novel agents, which were being increasingly used towards the end of the study.

In terms of the disease stage, patients in ISS stage I survive longer than patients in either stage II or III (median OS of 111, 66 and 45 months, respectively) after receiving an ASCT.[27] These findings were different in our study, with median OS times of 55 months, 82 months and 55 months for ISS stages I, II and III, respectively. These skewed findings do not reflect the true outcome in our study as the predicted survival time over a longer period could not be ascertained by statistical analysis due to the small number of patients in each staging group.

As is the case across the world, relapses are an impending occurrence in MM. 43.1% of the patients had relapsed after receiving an ASCT based on their last clinic visit

notes. More than half of the patients in the study (58.8%) were confirmed dead and a further five patients were presumed dead, but they were unable to be contacted telephonically.

When reviewed at six months post-ASCT, a significant proportion of the patients showed at least a PR (92.2%); 29.4% of the patients showed a CR and 31.4% a VGPR.

Three patients with HIV were transplanted. They were treated in the same manner as their HIV-negative counterparts, with the addition of combination antiretroviral therapy (cART). They had a mean CD4 count of 368 cells/µl and were all virologically suppressed prior to their ASCT. Two of them achieved a PR after their ASCT and one achieved a VGPR.

There were three patients with high-risk cytogenetics in our study. They all achieved a PR after ASCT and the differences in survival time when compared to their counterparts with low-/intermediate-risk risk cytogenetics was not statistically significant. However, the number of these patients was small and comparisons are therefore less meaningful.

4.12. Limitations of the study

Due to the retrospective nature of this study, there were a number of patients for whom data was missing, either due to inadequate record-keeping or missing information. There were also 10 patients (19.6 %) who were lost to follow up. The reason for this could be that these patients could no longer follow up at our centre due to financial or logistical reasons or may have died at home or at another medical facility closer to their residences.

This review looks at the patient profiles and outcomes in a very specific group of MM patients who received an ASCT at a single centre in SA. These findings should be treated with caution when compared to other Clinical Haematology centres in SA,

where this service may not be readily accessible or where the data from those centres may not have been published. The only other South African review that looked at ASCT in MM patients bears a number of differences as compared to our review. Although the patient cohort (42 patients) was almost comparable to ours, it was a prospective study and looked at the response in patients who were subjected to a sub-myeloablative dose of melphalan (100 mg/m^2) followed, six months later, by the standard 200 mg/m^2 dose. In terms of the disease response status after the myeloablative dose of melphalan with autologous stem cell rescue, 81% of the patients had achieved a CR or VGPR, as compared to 60.8% in our study.[71]

A large proportion of the patients did not have cytogenetic results available to risk-stratify their disease and guide management accordingly. Firstly, not all the BMAT procedures were done by our Clinical Haematology team or at our institution. A number of BMAT were done at other institutions before referral to our facility and unfortunately FISH analysis was not requested on those specimens. Secondly, BMAT samples are sometimes of poor quality, especially the marrow aspirate (which provides the medium for cytogenetics analysis). Thirdly, technical problems (e.g., not enough cells in metaphase) pose a hindrance to obtain adequate information on cytogenetics. Fourthly, FISH analysis is not routinely done or available on MM specimens at all the National Health Laboratory Services (NHLS) laboratories, or, if available, may not be done due to inadequate specialised staff at the time of sample submission.

The small number of patients included in our study was unfortunately a limiting factor against more robust and significant survival analyses to be carried out. For instance, we could not assess the survival outcomes when looking at the different induction chemotherapy groups and the different maintenance agents used post-ASCT due to too few numbers in the different groups.

CHAPTER 5: CONCLUSION

In this institution-based study of 51 newly diagnosed MM patients, ASCT use was associated with improvements in OS that persisted across the treatment eras that included the adoption of IMiDs towards the end of the second half of the study.

ASCT was increasingly utilised over the years as consolidation therapy, with more than half of the patients (64.7%) transplanted over the second half of the study period.

In keeping with older studies that included younger patients as being eligible for transplant, this study only included patients for transplant who were < 65 years of age. It would be recommended to consider older, fit individuals without significant comorbidities for ASCT in the future, based on the recommendations in the current literature.

The outdated VAD-based regimen still seems to be the standard for induction chemotherapy in our setting. It has been largely discontinued in first world countries owing to lower response rates as compared to the novel agents. This is a challenge in the South African public sector due to difficulties with acquiring 'state-of-the-art' therapy, including PIs and IMiDs (with the exception of thalidomide), in the public sector. The decision at our centre was to use thalidomide preferentially as maintenance therapy after ASCT rather than during induction as a result of the lack of other maintenance therapies. The introduction of novel agents will dramatically improve outcomes in our setting by impacting on the quality of the response to induction therapy, by allowing a greater number of patients to be offered ASCT as consolidation therapy and by giving us a choice of drugs which could be used as maintenance therapy.

This study did not show superiority of early ASCT over delayed ASCT. The delayed approach was offered to patients who started showing signs of disease progression after their induction chemotherapy. This approach may still be useful until the introduction of newer agents in the South African public sector, which will impact

on the quality of the response in our MM patient population, and allow for early intervention with an ASCT.

Tandem ASCT is generally not a practice at our institution and therefore no conclusions could be drawn regarding this practice.

As our patients generally presented late, with advanced stage disease, high-risk MM based on the cytogenetic profile did not seem to impart a worse prognosis in our patient population. However, the number of these patients was very small and, therefore, conclusions could not be made statistically.

Some limitations of the study stem from the fact that a number of patients were still risk-prognosticated based on the old Durie-Salmon staging system. Cytogenetic results, which were unfortunately not available on most of our patients, would have certainly impacted on treatment decisions regarding induction chemotherapy regimens.

In conclusion, ASCT in a South African public sector setting is advocated currently as it is feasible and is associated with improved survival. We require greater access to novel agents to impact on the initial induction response, which can be followed by an ASCT as a consolidation strategy, which has a clear benefit regarding OS. Maintenance therapy also needs to be optimised after an ASCT. This total optimisation of therapy in newly diagnosed MM will likely provide improved benefits to our patients who unfortunately could not be offered ‘state-of-the-art’ therapies at our public sector setting. This remains an ongoing challenge which needs to be addressed, as we continue to manage our MM patient population in the future.

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APPENDICES

Appendix A: HREC approval

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 5 July 2021

TITLE OF PROJECT:

Autologous stem cell transplantation in multiple myeloma at Chris Hani Baragwanath Academic Hospital.

UNIVERSITY: Witwatersrand

Principal Investigator: Dr V Rawoo.

Department: Internal Medicine

Supervisor/s : Prof M Patel

Permission Head Department (where research conducted): Yes

NHRD No

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

Recommended
(On behalf of the MAC)
2021/07/05

Approved/Not Approved
Hospital Management Date
Date: 2/7/2021

Appendix B: Extension of study approval

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

2023/01/27

Drs V Rawoo and A Lakha
School of Clinical Medicine
Department of Medicine
Clinical Haematology Unit
Medical School
University

Sent by e-mail to: girish.rawoo2011@gmail.com

Dear Dr Rawoo

Re: **Protocol Ref No:** M210739
Protocol Title: *Autologous stem cell transplantation in multiple myeloma at Chris Hani Baragwanath Academic Hospital*
Principal Investigators: Drs V Rawoo and A Lakha

Thank you for your letter of 2022/11/23, received here on 2023/01/26.

We have noted and approve of your proposal to extend your data collection window to include patient records up to 2021/12/31.

Thank you for keeping us informed.

Yours Sincerely

I. Burns

Mr I Burns
For the Human Research Ethics Committee (Medical)

CB Penny

Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

Appendix C: Revised International Staging System

Table 1. Standard Risk Factors for MM and the R-ISS

Prognostic Factor	Criteria
ISS stage	
I	Serum β_2-microglobulin $<$ 3.5 mg/L, serum albumin $\geq$ 3.5 g/dL
II	Not ISS stage I or III
III	Serum β_2-microglobulin $\geq$ 5.5 mg/L
CA by iFISH	
High risk	Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16)
Standard risk	No high-risk CA
LDH	
Normal	Serum LDH $<$ the upper limit of normal
High	Serum LDH $>$ the upper limit of normal
A new model for risk stratification for MM	
R-ISS stage	
I	ISS stage I and standard-risk CA by iFISH and normal LDH
II	Not R-ISS stage I or III
III	ISS stage III and either high-risk CA by iFISH or high LDH

Abbreviations: CA, chromosomal abnormalities; iFISH, interphase fluorescent in situ hybridization; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; R-ISS, revised International Staging System.

(Palumbo A, et al. 2015)

Appendix D: Durie-Salmon Staging System

Stage	Criteria	Tumor mass
I	All the following:	Low tumor mass
	Hb > 10 g/dL	< 0.6 x $10^{12}/m^2$
	Normal calcium	
	IgG < 5 g/dL; IgA < 3 g/dL	
	Monoclonal urinary protein < 4 g/24h	
	No or single bone lesion	
II	Between Stages I and II	Intermediate tumor mass
III	Any of the following:	High tumor mass
	Hb < 8.5 g/dL	> 1.2 x $10^{12}/m^2$
	Calcium < 12 mg/dL	
	IgG > 7 g/dL; IgA > 5 g/dL	
	Monoclonal urinary protein > 12 g/24h	
	Multiple osteolytic lesions, fractures	
Subclass	A – creatinine < 2 mg/dL	
	B – creatinine ≥ 2 mg/dL	

(Durie BG, et al. 1975)

Appendix E: International Myeloma Working Group (IMWG) Uniform Response Criteria

Category	Criteria
Stringent complete response (sCR)	CR as defined below PLUS: Normal SFLC ratio, AND Absence of clonal plasma cells in marrow by immunohistochemistry or flow cytometry
Complete response (CR)	Negative immunofixation on the serum and urine, AND Disappearance of any soft tissue plasmacytomas, AND ≤ 5% plasma cells in bone marrow
Very good partial response (VGPR)	Serum and urine M-protein detectable by immunofixation but not on electrophoresis, OR 90% reduction in serum M-protein plus reduction in 24h urinary M-protein by > 90% or to < 100 mg/24h
Partial response (PR)	≥ 50% reduction of serum M-protein, AND reduction in 24h urinary M-protein by ≥ 90% or to < 200 mg/24h, AND ≥ 50% reduction in the size of any plasmacytomas present at baseline (In place of M-protein criteria: If serum + urine M protein unmeasurable, ≥ 50% decrease in difference between involved and uninvolved SFLC levels, OR If serum + urine M-protein + SFLC assay also unmeasurable, a ≥ 50% reduction in marrow plasma cells is required, provided baseline bone marrow plasma cell percentage was ≥ 30%)
Stable disease (SD)	Not meeting criteria for CR, VGPR, PR or progressive disease
Progressive disease (PD)	At least ONE of the following: > 25% increase in serum M-protein in 3 months (absolute increase must be > 5 g/L), OR > 25% increase in urine M-protein in 3 months (absolute increase must be > 200 mg/24h), OR > 25% increase in the difference between involved and uninvolved SFLC levels (applicable only to patients without measurable serum and urine M- protein (absolute increase must be > 10 mg/dL), OR > 25% increase in bone marrow plasma cell percentage (absolute percentage must be > 10%), OR Development of new bone lesions or soft tissue plasmacytoma, OR Development of hypercalcaemia
Clinical relapse	Requires at least one of the following: Development of new bone lesions or soft tissue plasmacytoma, OR Increase in size of existing plasmacytomas or bone lesions, OR Any of the following attributable to myeloma: Development of hypercalcaemia, Development of anaemia (drop in Hb > 2 g/dL), Rise in serum creatinine
Relapse from CR	Requires at least one of the following: Reappearance of serum or urine M-protein by immunofixation or electrophoresis, OR Development of > 5% plasma cells in the bone marrow, OR Appearance of any other sign of progression (eg new plasmacytoma, new lytic bone lesion)

(Durie BG, et al. 2006)

Appendix F: Data collection sheet

Demographics

Study number	
Gender	Male: Female:
Age at presentation (yrs)	
Date of diagnosis (dd/mm/yyyy)	
Date of haematology referral (dd/mm/yyyy)	
Performance status (ECOG)	0: 1: 2: 3: 4:
Ethnic group	Black: White: Coloured/Mixed: Asian: Other:

History

SYMPTOMS	**YES/NO**	**DETAILS**
Bone pain		Site(s):
Pathological fracture(s)		Site(s):
Fever		
Significant weight loss		
Night sweats		
Fatigue/other symptoms of anaemia		
Bleeding		Site(s):
Hyperviscosity		
Plasmacytoma		Site(s):
Neuropathy/sensory changes		
Other		
CO-MORBIDITIES		
Hypertension		
Diabetes		
Cardiac/Pulmonary/Renal/Liver		
HIV		CD4: HIVVL:
Prior/current Tuberculosis:		
Other		

Examination

Sign	**Yes/No**	**Details**
Pallor		
Pedal oedema		
Jaundice		
Lymphadenopathy		Site(s):
Plasmacytoma/bone tenderness/fracture		Site(s):
Infection		
Thrombosis		
Hepatomegaly		cm below costal margin:
Splenomegaly		cm below costal margin:
Features of hyperviscosity		
Features of amyloidosis		
Cardiac failure		
Bleeding • Petechiae • Purpura • Ecchymoses • Mucosal		
Neurological deficit		
Other relevant clinical findings		

Imaging

	Date	Comments
CXR		
Skeletal survey		
Ultrasound		
CT scan		
MRI scan		

Bone Marrow findings

Aspirate	
Trephine	
Karyotype/cytogenetics (yes/no):	
• Aneu-/Hypo-/Hyperdiploid:	
• t(11;14)	
• t(4;14)	
• t(14;16)	
• t(14;20)	
• gain(1q21)	
• del(1p)	
• del(17p)	
• del(13q)/monosomy 13	
• TP53 mutation	
• Complex karyotype	
• Other	
Flow cytometry:	
• CD38	
• CD138	
• CD56	
• MUM-1	
• Ki-67	
• CD20	
• Kappa	
• Lambda	

Histology results (if applicable)

Date:_____

Findings:

_____

_____

Risk stratification:

1. Durie Salmon: _____
2. ISS/R-ISS:_____

Treatment: Specific

Modality	Details of treatment
Induction chemotherapy	Regimen/Dose: _____ Number of cycles:_____
Stem cell mobilisation:	Agent(s) used/dose:_____ CD34+ yield:_____
High-dose therapy:	Agent(s) used/dose:_____ No of doses: _____
Auto stem cell transplant	Date(s):_____ Single or tandem:_____ Second (salvage) transplant post-relapse (Yes/No): _____ Days taken for counts to recover spontaneously after stem cell infusion: • Platelets > $20x10^9$/l:_____ • ANC > $0.5x10^9$/l:_____
Maintenance chemotherapy	Agent(s)/Dose:_____ Duration:_____

Treatment: Supportive

Treatment	**Yes/No**
Allopurinol	
Analgesics	
Antibiotics	
Bisphosphonates	
Thromboprophylaxis	
Haemodialysis	
Blood products	
Radiotherapy	

Complications post-HDT/ASCT:

Early (within 2 weeks)	**Yes/No**	**Late (after 2 weeks)**	**Yes /No**
Mucositis		Mucositis	
Anaemia		Anaemia	
Neutropenia		Neutropenia	
Thrombocytopenia		Thrombocytopenia	
GIT symptoms		GIT symptoms	
Infections		Infections	
Bleeding		Bleeding	
		Alopecia	
		Infertility	

Response to treatment:

After induction chemotherapy		
• (sCR/CR/VGPR/PR/SD/PD/Relapse)		
• Duration		
After ASCT		
• (sCR/CR/VGPR/PR/SD/PD/Relapse)		
• MRD negativity		
• Duration		

Outcomes (as at last visit):

Living status (Alive/demised/Lost to follow-up)	
Disease response status	
Survival time (months from initial diagnosis)	
Relapse (duration post ASCT)	
Cause of death in non-survivors	
Lost to follow-up (last date seen and response status at last visit)	

Appendix G: Blood results

	Pre-chemo initiation	**Post-induction chemo**	**Post-ASCT**	**6 months post-ASCT**	**1 year post-ASCT**
DATE					
WCC ($x10^9$/l)					
Haemoglobin (g/dl)					
Haematocrit (%)					
MCV (fl)					
Platelets ($x10^9$/l)					
Neutrophils ($x10^9$/l)					
Lymphocytes ($x10^9$/l)					
Monocytes ($x10^9$/l)					
Basophils ($x10^9$/l)					
Eosinophils ($x10^9$/l)					
Reticulocytes (%)					
RPI					
Sodium (mmol/l)					
Potassium (mmol/l)					
Chloride (mmol/l)					
Carbon dioxide (mmol/l)					
Urea (mmol/l)					
Creatinine (µmol/l)					
Calcium (mmol/l)					
Corrected Calcium(mmol/l)					
Magnesium (mmol/l)					
Phosphate (mmol/l)					
Total bilirubin (µmol/l)					
Conjugated bilirubin(µmol/l)					
Total Protein (g/l)					
Albumin (g/l)					
Alkaline phosphatase (U/l)					
Gamma glutamyl transferase (U/l)					
Alanine transaminase (U/l)					
Aspartate transaminase (U/l)					

Iron (µmol/l)					
Transferrin (g/l)					
Transferrin saturation (%)					
Ferritin (µg/ml)					
Vitamin B12 (pmol/l)					
Serum Folate (µg/l)					
D-dimers (µg/ml)					
Uric acid (mmol/l)					
Beta-2-microglobulin ((mg/l)					
Lactate dehydrogenase (U/l)					
Paraprotein (g/l)					
IgG (g/l)					
IgA (g/l)					
IgM (g/l)					
Serum FLC (mg/l)					
K					
Λ					
Ratio					
HIV status					
CD4 count (cells/µl)					
HIVVL (copies/ml)					

Appendix H: Turnitin report

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WITS UNIVERSITY

31 May 2024

The Chair

Postgraduate Studies Committee

Faculty of Health Sciences

University of the Witwatersrand

Re: Turn-it-in report: Dr Vedanand Rawoo (student number 2320971) – MMed: Autologous Stem Cell Transplantation in Adult Multiple Myeloma Patients at Chris Hani Baragwanath Academic Hospital

I have reviewed the Turn-it-in report of Dr Rawoo's MMed Research Report. The report identifies a similarity index of 29%. Much of this similarity relates to recurring terminology, classifications, staging systems and definitions which are standardized. The other information which bears a similarity has been appropriately and correctly referenced.

Thanking you

Yours sincerely

Moosa Patel MBChB, FCP(SA), MMed(Wits), FRCP(Lond.), PhD(Wits)

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