A LABORATORY BASED RETROSPECTIVE STUDY OF PLASMA CELL MYELOMA IN THE PUBLIC SECTOR OF SOUTH AFRICA FROM 2017 TO 2019

Dr Bradley Thomas Wilding, MBBCh (Wits), Dip HIV Man (SA)

Supervisors:
Prof. Jaya George, BMBCH; DTM&H; MSc; FC Path (Clin); PHD
Dr Tracey Wiggill, MBBCh; MMed (Haem)
Dr Ngalula, Kone MBChB; FC Path (Chem)

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

Johannesburg, 2024

DECLARATION

I Bradley Thomas Wilding declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the branch of Pathology (Clinical pathology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature: 

Date: 21/02/2024

ABSTRACT

Background

Plasma cell myeloma is a haematological malignancy characterized by clonal proliferation of plasma cells. This malignancy is frequently associated with the production of a monoclonal protein in either serum and / or urine, referred to as an M protein, which is used as a screening test for patients. Patients are then further investigated to assess if they meet the International Myeloma Working Group (IMWG) diagnostic criteria for plasma cell myeloma. There is limited literature describing plasma cell myeloma in South Africa, particularly in people living with HIV.

Objective

The primary objective of this study was to describe plasma cell myeloma in patients diagnosed in the public sector of South Africa over a three-year period. The secondary objective was to compare demographic features (age, sex) and diagnostic criteria, between the myeloma patients living with HIV and the HIV negative myeloma patients.

Methods

A retrospective analysis was performed on data from 4518 patients who had a positive immunofixation on serum and / or urine from public sector hospitals, between 2017 and 2019. A total of 718 of the 4518 patients met the laboratory criteria for plasma cell myeloma and were included in the analysis. Demographics (age, sex) and laboratory investigations used in the diagnostic criteria for plasma cell myeloma were analysed and statistically compared across the different HIV status of patients.

Results

Plasma cell myeloma patients presented at a mean age of 59.46 years with a female to male ratio of 1.2:1. In the patients that met the diagnostic criteria the most common end-organ damage present was anaemia in 77.16% patients and the most common biomarker of malignancy was a bone marrow trephine biopsy plasma cell percentage ≥60% in 55.71% patients. IgG isotype was the most common paraprotein detected on serum immunofixation in 58.5% of the patients. Kappa was the most common Bence-Jones protein detected in 27.16% of patients which was 1.76 times more common than lambda Bence-Jones protein.

People living with HIV were younger 55.11 (±9.79) as compared to their HIV negative counterparts (*p* value 0.010). No other statistically significant difference was noted when comparing HIV status groups.

Conclusion

In conclusion, this study described the demographics, laboratory investigations and diagnostic features of plasma cell myeloma patients diagnosed in the South African public sector from 2017 to 2019. We found that people living with HIV were diagnosed at younger age when compared to their HIV negative counterparts.

ACKNOWLEDGEMENTS

I would like to thank the following people for everything they have done to assist me in completing this masters project:

1. My supervisors Prof. Jaya George, Dr Tracey Wiggill and Dr Ngalula Kone for their continued advice and support throughout this project.
2. My collaborators Naseem Cassim, Michael Mapunda and Victor Olago for their assistance with the data cleaning and text mining.
3. Prof. Nigel Crowther for the assistance with the statistical tests required to analyze the data
4. Mrs. Stacey Marie Jackson for the assistance with excel functions and excel formulas for the cleaning and analyzing of the data
5. The National Health Laboratory Service (NHLS) for the use of the data

TABLE OF CONTENTS

DECLARATION 2

ABSTRACT 3

ACKNOWLEDGEMENTS 5

LIST OF FIGURES 7

LIST OF TABLES 8

LIST OF ABBREVIATIONS 9

NOTE ON THE FORMAT OF THIS RESEARCH REPORT 10

INTRODUCTION AND LITERATURE REVIEW 11

Background 11
Plasma cell myeloma in the South African 12
Human Immunodeficiency Virus and plasma cell myeloma 13
Objectives 13

METHODS 14

Materials and Methods 14
Data analysis 16
Ethical considerations 16

RESULTS 16

Immunofixation results 17

DISCUSSION 22

REFERENCES 26

LIST OF FIGURES

Figure 1: Study population used for analysis.

Figure 2: Serum immunoglobulin paraproteins of study population.

Figure 3: Urine immunofixation findings of study population.

LIST OF TABLES

Table 1: Diagnostic criteria for plasma cell myeloma.

Table 2: Racial distribution of plasma cell myeloma patients from 2017-2019 (South African National Cancer Registry statistics).

Table 3: Demographics of study population including HIV status groups.

Table 4: Age and laboratory investigations of study population including HIV status groups.

Table 5: Plasma cell diagnostic criteria of study population including HIV status groups.

Table 6: Serum immunoglobin paraproteins of study population including HIV status groups.

Table 7: Urine findings of study population including HIV status groups.

LIST OF ABBREVIATIONS

CDW – Central data warehouse

HIV – Human Immunodeficiency Virus

IMWG – International Myeloma Working Group

LIS – Laboratory information system

MGUS – monoclonal gammopathy of undetermined significance

NCR – National Cancer Registry

NHLS – National Health Laboratory Service

PCM – Plasma cell myeloma

PLWH – People living with HIV

SFLC – Serum free light chains

USA – United States of America

WHO – World Health Organization

NOTE ON THE FORMAT OF THIS RESEARCH REPORT

Before undertaking of this project, a protocol including a literature review and methodology plan was submitted to and approved by the post graduate committee from the School of Pathology at the University of the Witwatersrand.

This project was also approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and a copy of the approval letter can be found in the appendices.

The format of this Research Report is as stipulated by the Committee for Postgraduate studies, Faculty of Health Sciences at the University of the Witwatersrand. The format of the submissible article may differ from the rest of the research report. This is to comply with the author's guidelines of PloS One journal, to which it is intended to be submitted.

INTRODUCTION AND LITERATURE REVIEW

Background

Plasma cell myeloma (PCM) is a clonal proliferation of plasma cells with a frequently produced monoclonal immunoglobulin referred to as an M protein in the serum and / or urine and, is often associated with end organ damage (1). PCM is part of the group of plasma cell neoplasms (1). It is usually preceded by a pre-malignant phase known as monoclonal gammopathy of undetermined significance (MGUS) (2) with a risk of progression to PCM of 0.5-1% per year (3). There is also a more advanced premalignant intermediate stage between MGUS and PCM known as smouldering plasma cell myeloma, which presents with a risk of progression to PCM of around 10% per year for the first 5 years (3). The diagnostic criteria for PCM were revised in 2014 by the International Myeloma Working Group (IMWG) (see table 1) (3).

Table 1: Diagnostic criteria for plasma cell myeloma.

Adapted from the International Myeloma Working Group (IMWG) diagnostic criteria (3)

Bone marrow clonal plasmacytosis of ≥ 10% or biopsy-proven bony or extramedullary plasmacytoma AND any one or more of the following myeloma defining events:
End-organ damage attributable to the underlying plasma cell proliferative disorder • Hypercalcaemia: serum calcium >2.75 mmol/L or serum calcium >0.25 mmol/L higher than the upper reference limit • Renal insufficiency: creatinine clearance of <40 ml per minute or a serum creatinine >177 µmol/L • Anaemia: haemoglobin level of >2.0 g/dl below the lower limit of normal or a haemoglobin value <10 g/dl • Bone lesions: ≥1 osteolytic lesions on skeletal radiography, computerized tomography (CT) scan or positron emission (PET) CT scan ≥1 of the following biomarkers of malignancy • Bone marrow clonal plasmacytosis of ≥60% • Involved:uninvolved serum free light chain ratio (SFLC) ≥100 • >1 focal lesion on magnetic resonance imaging (MRI)

According to the World Health Organization (WHO), PCM is responsible for about 1% of all malignant neoplasms and ranges between 10-15% of all haematological malignancies (1). PCM is more common in males than females and is twice as prevalent in Black African patients when compared to

White patients, with more marked discrepancy noted in younger patients according to both the WHO and a population based study performed by Waxman *et al* in the United States of America (USA) (1,4). It affects the elderly population with a median age at presentation of 66 years according to a study in the USA (5). However, two local studies conducted at the Chris Hani Baragwanath Academic Hospital (n=170) and in the Eastern Cape province (n=464) showed the mean age of diagnosis to be younger (61.4 years and 61 years respectively) (6,7). This suggests that PCM may occur at a younger age in South Africa. Worldwide, the incidence rate of PCM has increased by 126% from 1990 to 2016 with a worldwide age-standardized incidence rate of 2.1 per 100 000 persons worldwide compared to between 1.6-2.1 in South Africa (8).

Plasma cell myeloma in the South African

According to the South African National Cancer Registry (NCR) statistics for 2017 to 2019, there were a total of 1198 new PCM cases diagnosed histologically, including 588 females (49.1%) and 610 males (50.9%). Over the three-year period, PCM accounted for an average of 0.46% of all female cancers, with an average incidence rate of 0.66 adjusted cases per 100 000/year and 0.50% of all male cancers, with an average incidence rate of 0.72 adjusted cases per 100 000/year (9–11). The racial distribution of PCM according to the NCR statistics between 2017 to 2019 is shown in the table below (see table 2):

Table 2: Racial distribution of plasma cell myeloma patients from 2017-2019 (South African National Cancer Registry statistics) (9–11)

Race	**Female (percentage)**	**Male (percentage)**
Asian	18 (3.06%)	14 (2.30%)
Black African	294 (50.00%)	275 (45.08%)
Coloured	65 (11.05%)	67 (10.98%)
White	198 (33.67%)	241 (39.51%)
Unknown	13 (2.21%)	13 (2.13%)
TOTAL	**588 (100%)**	**610 (100%)**

The highest percentage of cases in the South African population occurred within the Black African population. However, this data has not been adjusted for the national population racial distribution and may reflect under reporting, reporting bias and differential access to health care in South Africa in different population groups. The highest incidence occurred in females above the age of 55 and in males above the age of 50. These incidences appear to be similar to other local studies (6,7). In a

review of 1027 patients with PCM attending Mayo Clinic in Minnesota USA, it was shown that 2% and 8% of patients were below the age of 40 and between 40 and 50 years respectively (5). According to the NCR for South Africa, there were on average 3.84% and 10.27% of patients with PCM below the age of 40 and between the ages of 40 and 50 years respectively, between 2017 and 2019 (9–11). This was also noted in the study performed at Chris Hani Baragwanath Academic hospital with 7.1% and 5.9% of patients diagnosed with PCM being below the age of 40 and between the ages of 40-50 years respectively (6). This suggests that a greater proportion of patients in South Africa are diagnosed with PCM at a younger age.

Human Immunodeficiency Virus and plasma cell myeloma

There is limited literature describing patients who are Human Immunodeficiency Virus (HIV) positive with plasma cell myeloma and the evidence to date is conflicting. People living with HIV (PLWH) have been shown to present with plasma cell disorders (MGUS, plasmacytoma and PCM) at a much younger age, with an average age of 32 years at diagnosis, for documented plasma cell neoplasms in the USA (12). In a local study by De Groot *et al*., PCM occurred at a younger age with the median age of 50,5 years old in PLWH. These patients were also found to have less renal impairment and fewer lytic lesions compared to HIV negative patients (13).

However, a study on 170 patients with PCM attending Chris Hani Baragwanath Academic Hospital, showed that the HIV seropositive rate appeared lower than the hospital population matched for age, suggesting a lack of association between HIV and PCM (6). In a recent study by Sengayi-Muchengeti *et al* that looked at cancers associated with HIV infection in Black African South African patients, PCM was shown to have no association with HIV (14).

Objectives

The primary objective of this study was to describe PCM in patients diagnosed in the public sector of South Africa over a three-year period. The secondary objective was to compare demographic features (age, sex) and diagnostic criteria, between the PCM patients living with HIV and the HIV negative myeloma patients.

METHODS

Materials and Methods

This study was a retrospective, descriptive, cross-sectional analysis using data extracted from the National Health Laboratory Service (NHLS) laboratory information system (LIS -TrakCare) and the central data warehouse (CDW), for all patients who attended a public sector South African hospital over a three-year period from 1 January 2017 to 31 December 2019. Figure 1 describes the initial patients' identification and summarizes the study's inclusion and exclusion criteria. As recommended (15), immunofixation on either serum or urine was used as the first test to identify possible PCM patients (16). There were 7907 patients who, for various clinical reasons, had at least one immunofixation performed on either blood and / or urine during the study period. After reviewing the initial results, 4518 patients who had a serum and / or urine M protein detected were selected and the remaining patients were excluded, as their immunofixation was either negative, polyclonal or showed oligoclonal banding. Only 1564 patients had either a bone marrow trephine biopsy or bone marrow flow cytometry performed, which is required for the diagnosis and to prove clonality of the plasma cells, using the diagnostic criteria in Table 1. A further 2954 patients were excluded at this stage, as no bone marrow was performed and therefore, not meeting the diagnostic criteria. The bone marrow results were reviewed for the 1564 patients, of which 813 patients were confirmed as having PCM by meeting the diagnostic criteria from a laboratory point of view. A further 751 patients who displayed MGUS, smouldering myeloma or a malignancy other than multiple myeloma were excluded, as they did not meet the diagnostic criteria for this study.
Of the 813 patients identified as having PCM, 26 were reported to be in remission with 69 patients already diagnosed previously. These 95 patients were therefore also excluded, for being diagnosed out of the study period.
A total of 718 patients, meeting the PCM diagnostic criteria, constituted the final study population for this 3-year period study.

Patients' data was extracted using the unique patient identifier. The following information was retrieved and reviewed: age, sex, race, province, haemoglobin, calcium, creatinine, estimated glomerular filtration rate (eGFR), bone marrow aspirate, bone marrow flow cytometry, bone marrow trephine biopsy, serum free light chains (SFLC), HIV serology, CD4 count and HIV viral load.
The time period used for patient results were as follows:

- The haemoglobin, calcium, creatinine and eGFR results used were the ones closest to the date of the immunofixation result which, were less than a week prior to, or after the immunofixation result.
- SFLC results were obtained closest to the date of the immunofixation result and were not older than three months prior to, or after the immunofixation result.
- Bone marrow aspirate, bone marrow flow cytometry and bone marrow trephine/histology results used were not older than one year prior to or, after the immunofixation result.
- HIV status was established based on the following:
    - A positive HIV serology result and/or detectable viral load and/or mention of positive HIV status on the request form with a viral load or CD4 count.

All results were reviewed for each patient and selected based on them meeting the IMWG diagnostic criteria for PCM (3).

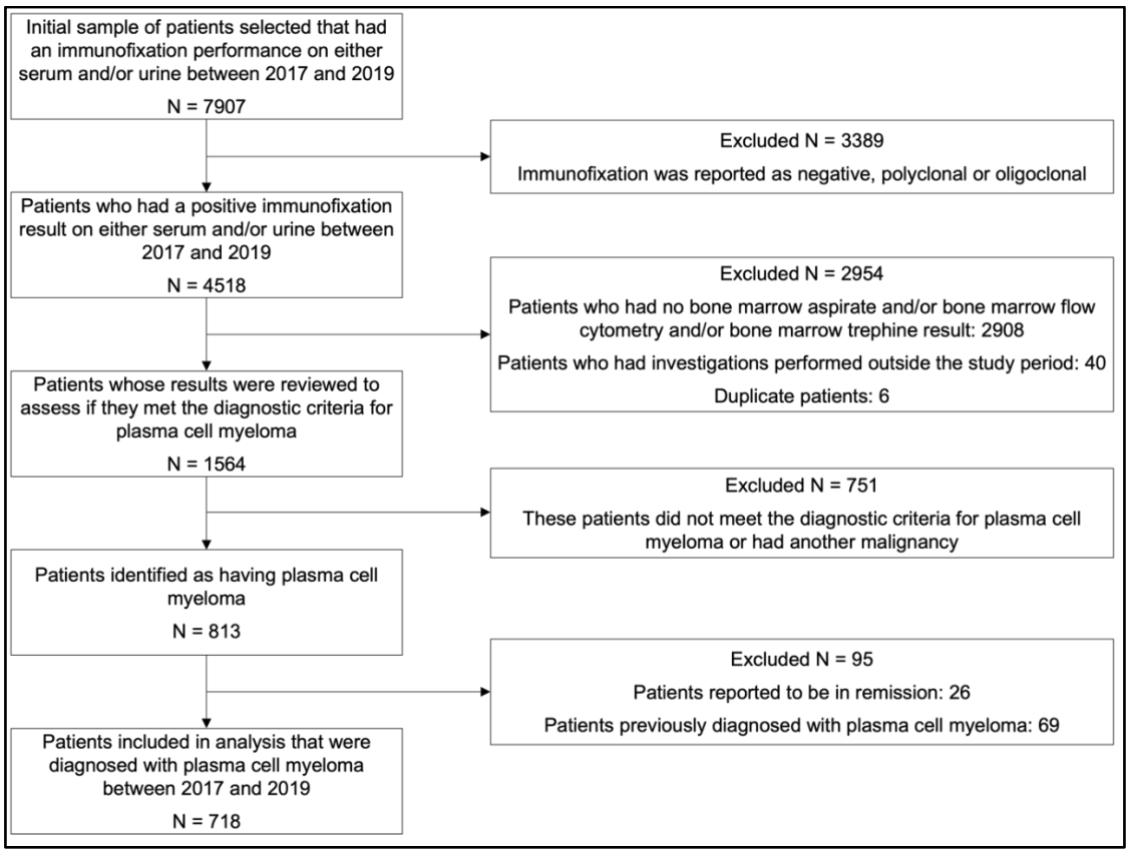

Figure 1: Study population used for analysis.

Data analysis

Statistical analysis was performed using GraphPad Prism version 9.5.1 for macOS, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

The categorical variables are represented as frequencies and percentages. The continuous variables are presented as means and standard deviations or medians and interquartile ranges depending on whether the data is parametric. When comparing PLWH, HIV negative and HIV unknown patients, the *p*-values were determined using an unpaired t-test and a one-way analysis of variance (ANOVA) test for the normally distributed data. A Mann-Whitney test and a Kruskal-Wallis test were performed on the non-parametric data. A chi-squared test was performed on the categorical variables. Statistical significance was defined assuming a two sided *p*-value of <0.05.

Microsoft Excel was used for the pivot tables and frequency graphs on the immunofixation data.

Ethical considerations

The study protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand (protocol reference number: M200627).

RESULTS

Of the 718 patients diagnosed between 2017 and 2019 there were 71 (9.89%) PLWH, 459 (63.93%) patients were HIV negative and 188 (26.18%) patients whose HIV status was unknown. In the whole group most patients were from Gauteng (Table 3). Race was only recorded for 50 out of the 718 (6.96%) patients included for analysis.

PCM was found proportionally more in female patients (54.74%) compared to male patients (45.40%) equating to a ratio of 1.2:1. In PLWH there were slightly more males (50.70%) than females (49.30%). When comparing gender across HIV status there was a statistically significant difference in gender (*p*-value 0.049) which was predominantly due to the large difference in HIV-negative to HIV unknown patients (*p*-value 0.024) (Table 3).

Table 4 illustrates the age and laboratory investigations of the study population. The overall average age at diagnosis was 59.46 years (±10.7). The average haemoglobin level was 8.52 g/dL (±2.42) and the median and interquartile range for calcium and creatinine were 2.36 mmol [2.16-2.71] and 120 µmol/L respectively [77-305.5]. PLWH were younger 55.11 (±9.79) years vs HIV-negative 58.56 (±10.55) years, while HIV unknown patients had the highest mean age of 63.33 (±10.29) years. There

was no statistically significant difference noted in haemoglobin, calcium and creatinine levels across the various HIV status groups (Table 4).

Anaemia was the most common feature of end organ damage with 77.16% of patients having a haemoglobin concentration of <10 g/dL or >2 g/dL below the reference interval for age and sex. Renal insufficiency was present in 35.65% of patients and hypercalcaemia was the least common end organ damage with only 20.06% patients meeting the diagnostic criteria. Lytic lesions were mentioned in the history provided, for 29.81% of patients. However, it is uncertain if this was based on radiography, CT, PET CT or MRI (Table 5).

Bone marrow trephine biopsy showed that 55.71% of patients met the diagnostic criteria of having a plasma cell percentage of ≥60% and, 24.79% of patients met the diagnostic criteria with a SFLC involved:uninvolved ratio of ≥100. The majority of patients did not have a SFLC test. When comparing SFLC across HIV status groups, proportionately more HIV negative had a diagnostic result (*p* value 0.0006), which was due to a much larger percentage of patients not having a SFLC performed in the HIV unknown group. Of note, there was a large proportion of patients (65.46%) who did not meet the diagnostic criteria for clonality on flow cytometry of bone marrow aspirate, and this was predominantly because flow cytometry was not performed (Table 5).

Immunofixation results

The serum findings showed that the most common immunoglobulin (M-protein) was the IgG isotype (58.5%) followed by the IgA isotype (18.11%) and then light chain disease (15.32%). Associated with IgG heavy chain, the kappa light chain was more frequent (39.28%) than the lambda light chain (19.22%). Thirty-eight patients (5.29%) had no immunofixation done on serum during the study period, and were identified by urine findings. Across HIV status, the M-protein types that were found appeared to be distributed consistently across the groups (Table 5 and Figure 2).
The most common Bence-Jones protein detected in urine were of the kappa type (27.16%). There was also a significant number of patients who had a myeloma protein present in their urine (31.75%). A large proportion of patients did not have a urine immunofixation performed (41.09%). The urine findings showed, once again, a fairly consistent distribution across the HIV status groups (Table 6 and Figure 3).

Table 3: Demographics of study population including HIV status groups.

	Total patients (n = 718)	HIV-positive (n = 71)	HIV-negative (n = 459)	HIV-unknown (n = 188)	HIV status compared	HIV pos vs neg	HIV pos vs unkn	HIV neg vs unkn
	n (%)	n (%)	n (%)	n (%)	*p* value	*p* value	*p* value	*p* value
Gender:								
Female	393 (54.74)	35 (49.30)	241 (52.51)	117 (62.23)	0.049	0.614	0.059	0.024
Male	326 (45.40)	36 (50.70)	218 (47.49)	71 (37.77)				
Province:								
Gauteng	314 (43.73)	35 (49.30)	194 (42.27)	85 (45.21)				
Western Cape	208 (28.97)	12 (16.90)	155 (33.77)	41 (21.81)				
KwaZulu-Natal	68 (9.47)	8 (11.27)	32 (6.97)	28 (14.89)				
Free State	48 (6.69)	10 (14.08)	31 (6.75)	7 (3.72)				
Eastern Cape	39 (5.43)	3 (4.23)	21 (4.58)	15 (7.98)				
North West	15 (2.09)	1 (1.41)	9 (1.96)	5 (2.66)				
Northern Cape	10 (1.39)	0 (0.00)	9 (1.96)	1 (0.53)				
Limpopo	10 (1.39)	1 (1.41)	4 (0.87)	5 (2.66)				
Mpumalanga	6 (0.84)	1 (1.41)	4 (0.87)	1 (0.53)				

Table 4: Age and laboratory investigations of study population including HIV status groups.

	Total patients (n = 718)		HIV-positive (n = 71)		HIV-negative (n = 459)		HIV-unknown (n = 188)		HIV status compared	HIV pos vs neg	HIV pos vs unkn	HIV neg vs unkn
	n		n		n		n		*p* value	*p* value	*p* value	*p* value
Age (years):	716	59.46 ± 10.7	71	55.11 ± 9.79	459	58.56 ± 10.55	186	63.33 ± 10.29	<0.0001	0.010	<0.0001	<0.0001
Haemoglobin (g/dl):	702	8.52 ± 2.42	69	8.27 ± 2.86	449	8.54 ± 2.45	184	8.57 ± 2.19	0.642	0.394	0.361	0.874
Calcium (mmol/L):	658	2.36 (2.16-2.71)	64	2.37 (2.17-2.93)	426	2.37 (2.17-2.71)	168	2.33 (2.14-2.60)	0.369	0.555	0.244	0.239
Creatinine (μmol/L):	678	120 (77-305.5)	69	145 (74-437)	431	125 (77-315)	180	114.5 (78.25–253.5)	0.601	0.832	0.460	0.351

Data is represented as mean ±SD or median (interquartile range).

Table 5: Plasma cell diagnostic criteria of study population including HIV status groups.

	Total patients (n = 718)	HIV-positive (n = 71)	HIV-negative (n = 459)	HIV-unknown (n = 188)	HIV status compared	HIV pos vs neg	HIV pos vs unkn	HIV neg vs unkn
	n (%)	n (%)	n (%)	n (%)	*p* value	*p* value	*p* value	*p* value
Bone marrow trephine biopsy:								
Does not meet diagnostic criteria	46 (6.41)	3 (4.23)	28 (6.10)	15 (7.97)				
10-60% clonal plasma cells	272 (37.88)	32 (45.07)	164 (35.73)	76 (40.42)	0.336	0.298	0.517	0.283
≥60% clonal plasma cells	400 (55.71)	36 (50.70)	267 (58.17)	97 (51.60)				
Flow cytometry / bone marrow aspirate:								
Does not meet diagnostic criteria	470 (65.46)	46 (64.79)	296 (64.49)	128 (68.01)				
<10% clonal cells	26 (3.62)	2 (2.82)	18 (3.92)	6 (3.19)	0.914			0.601
10-60% clonal plasma cells	183 (25.49)	19 (26.76)	122 (26.58)	42 (22.34)				
≥60% clonal plasma cells	39 (5.43)	4 (5.63)	23 (5.01)	12 (6.38)				
Lytic lesion:								
No mention in history provided	391 (54.46)	35 (49.30)	259 (56.43)	97 (51.60)				
No lytic lesion	25 (3.48)	3 (4.23)	17 (3.70)	5 (2.66)	0.677	0.530	0.733	0.550
Lytic lesion mentioned in history provided	214 (29.81)	21 (29.58)	131 (28.54)	62 (32.98)				
Mention of other lesion. fracture or pathological fracture	88 (12.26)	12 (16.90)	52 (11.33)	24 (12.77)				
Calcium:								
No result	60 (8.36)	7 (9.86)	33 (7.12)	20 (10.64)				
Does not meet the diagnostic criteria	514 (71.59)	45 (63.38)	337 (73.42)	132 (70.21)	0.307	0.214	0.409	0.345
Meets the diagnostic criteria	144 (20.06)	19 (26.76)	89 (19.39)	36 (19.15)				
Haemoglobin:								
No result	16 (2.23)	2 (2.82)	10 (2.18)	4 (2.13)				
Does not meet the diagnostic criteria	148 (20.61)	13 (18.31)	100 (21.79)	35 (18.62)		0.770		0.663
Meets the diagnostic criteria	554 (77.16)	56 (78.87)	349 (76.03)	149 (79.25)				
Creatinine:								
No result	40 (5.57)	4 (5.63)	28 (6.10)	8 (4.26)				
Does not meet the diagnostic criteria	422 (58.77)	39 (54.93)	265 (57.73)	118 (62.77)	0.684	0.867	0.509	0.413
Meets the diagnostic criteria	256 (35.65)	28 (39.44)	166 (36.17)	62 (32.98)				
eGFR:								
No result	65 (9.05)	6 (8.45)	44 (9.59)	15 (7.98)				
Does not meet the diagnostic criteria	357 (49.72)	32 (45.07)	227 (49.46)	98 (52.13)	0.825	0.679	0.588	0.739
Meets the diagnostic criteria	296 (41.23)	33 (46.48)	188 (40.96)	75 (39.89)				
Serum free light chains (SFLC):								
No result	315 (43.87)	30 (42.25)	179 (39.00)	106 (56.38)				
Does not meet the diagnostic criteria	225 (31.34)	28 (39.44)	151 (32.90)	46 (24.47)	0.0006	0.208	0.0493	0.0003
Meets the diagnostic criteria	178 (24.79)	13 (18.31)	129 (28.10)	36 (19.15)				

Flow cytometry / bone marrow aspirate - plasma cells if found to be clonal were quantified (%) using the amount of plasma cells seen on the bone marrow aspirate as plasma cells are often lost during flow cytometry due to their size. Where there is no *p*-value this is due to low numbers within one the diagnostic criteria category and therefore could not be accurately calculated.

Table 6: Serum immunoglobin paraproteins of study population including HIV status groups.

	Total patients (n = 718)	HIV-positive (n = 71)	HIV-negative (n = 459)	HIV-unknown (n = 188)
	n (%)	n (%)	n (%)	n (%)
IgG Kappa	282 (39.28)	31 (43.66)	174 (37.91)	77 (40.95)
IgG Lambda	138 (19.22)	9 (12.68)	88 (19.17)	41 (21.81)
IgA Kappa	82 (11.42)	10 (12.68)	56 (12.20)	17 (9.04)
IgA Lambda	48 (6.69)	4 (5.63)	32 (6.97)	12 (6.38)
IgM Lambda	1 (0.14)	0 (0)	1 (0.22)	0 (0)
IgD Lambda	2 (0.28)	0 (0)	1 (0.22)	1 (0.53)
Kappa	55 (7.66)	4 (5.63)	41 (8.93)	10 (5.32)
Lambda	55 (7.66)	5 (7.04)	37 (8.06)	13 (6.91)
Other	17 (2.37)	4 (5.63)	8 (1.74)	5 (2.66)
No serum immunofixation	38 (5.29)	5 (7.04)	21 (4.58)	12 (6.38)

Other - includes patients who either had more than one type of heavy chain and / or light chain present or had just a heavy chain present with no associated light chain.

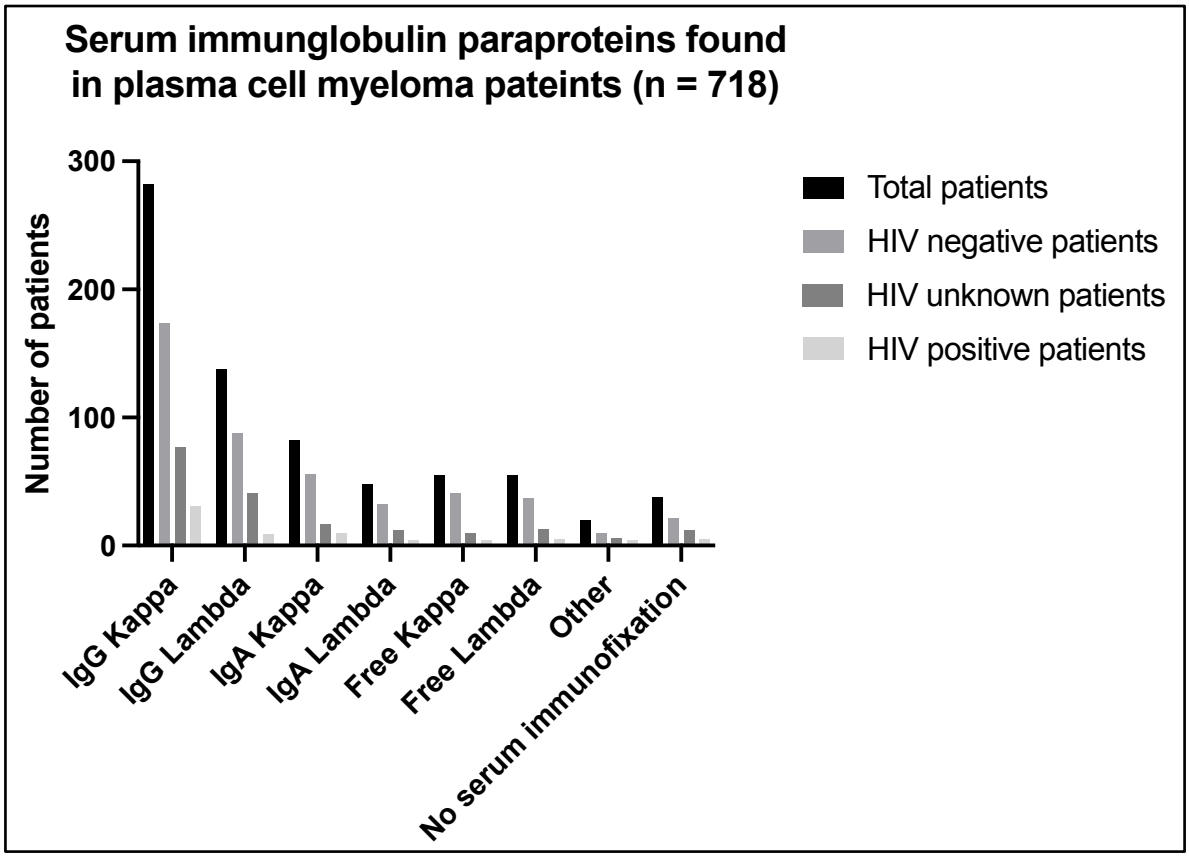

Figure 2: Serum immunoglobulin paraproteins of study population.
Other – includes the rare types IgM lambda and IgD lambda as well as patients who either had more than one type of heavy chain and / or light chain present or had just a heavy chain present with no associated light chain.

Table 7: Urine findings of study population including HIV status groups.

	Total patients (n = 718)	HIV-positive (n = 71)	HIV-negative (n = 459)	HIV-unknown (n = 188)
	n (%)	n (%)	n (%)	n (%)
Kappa BJP	195 (27.16)	20 (28.17)	128 (27.89)	47 (25.00)
Lambda BJP	111 (15.46)	14 (19.72)	73 (15.90)	24 (12.77)
Mixed Kappa and Lambda BJP	3 (0.42)	2 (2.82)	1 (0.22)	0 (0)
No BJP detected	114 (15.88)	8 (11.27)	81 (17.65)	25 (13.30)
Myeloma Protein detected	228 (31.75)	23 (32.39)	147 (32.03)	58 (30.85)
Myeloma Protein not detected	195 (27.16)	21 (29.58)	136 (29.63)	38 (20.21)
No urine immunofixation	295 (41.09)	27 (38.03)	176 (38.34)	92 (48.94)

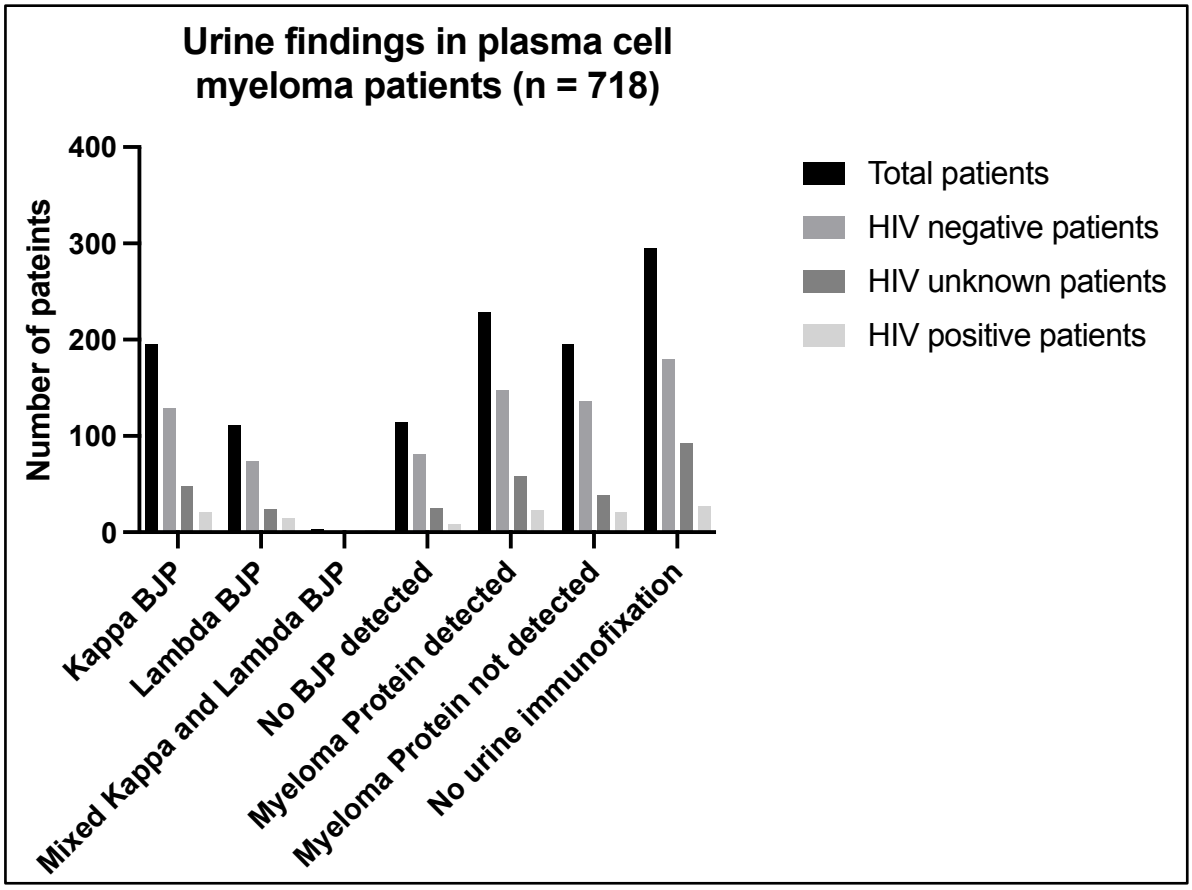

Figure 3: Urine immunofixation findings of study population.

DISCUSSION

The purpose of this study was to describe PCM across the public sector of South Africa as well as to compare the features across HIV status. This study found that more females were diagnosed (54.74%) than males (1.2:1 ratio) and, myeloma was diagnosed at an earlier age in PLWH.

Our finding of a higher incidence in females is different to the NCR data, that shows a male predominance (9–11). A recently published paper by Chili *et al* looking at plasma cell myeloma in a tertiary hospital in KwaZulu-Natal also found female predominance of 54% in their study cohort, in keeping with our study (17). Another local study by Naidoo *et al*. looking at the prevalence of electrophoresis testing at a tertiary hospital in South Africa, showed a female to male ratio for serum and urine immunofixation of 1.1:1 which may explain our findings as immunofixation was the test used to identify patients (18). Another possible explanation may be better health seeking behavior in female patients. A recently published study by Govendar *et al*, showed twice as many females attended primary health care facilities (19).

The mean age of diagnosis was slightly lower than what was shown in local studies conducted at the Chris Hani Baragwanath Academic Hospital (n=170) and in the Eastern Cape province (n=464), at 61.4 years and 61 years respectively (6,7). This was however, slightly older than the median age of 54 years found in a study in India by Jacob *et al*, a country with similar limited resource settings (20). In both studies, the diagnosis was made at a much younger age than that reported in the USA (median of 66 years) (5). This suggests that PCM is being diagnosed at a younger age in limited resource settings, possibly due to the higher HIV prevalence in lower income countries.

When comparing PLWH to HIV negative patients the mean age of diagnosis was significantly lower in PLWH, compared with the HIV negative population. These findings are consistent with the study by De Groot *et al* which showed a median age of 50.5 years in PLWH, although this was a small study of only 16 PLWH (13). A recent abstract by Xiao *et al*, showed a mean age of diagnosis to be 61.5 years in 14 PLWH (21). In a meta-analysis published by Grulich *et al* in 2007 which looked at the incidence of cancers in patients with HIV/AIDS compared to those with post solid-organ transplantation patients who were immunosuppressed, it was shown that PCM had an increased incidence in both groups of patients with a study standardized incidence ratio (95% confidence intervals) of 2.71 (2.13-3.44) for PLWH and 3.12 (2.13-4.57) for transplant patients (22). This implies that immunocompromised individuals may be at higher risk of acquiring PCM. HIV has been implicated in pathogenic mechanisms that accelerate the PCM carcinogenic process through immune activation, inflammation, and immunosuppression, hence hastening the aging process of people with PCM diagnoses (23).

There were 480 more patients diagnosed with PCM on histology from 2017-2019 by the NCR in South Africa when compared to our study. This is because NCR data is captured from both the public and private sector (9–11) and according to the WHO 68% of the South African population do not use any private health care (24). A further 16% rely on the public sector for hospital care, using the private sector for primary health care only (24). This, as well as the limitations listed below, may partially explain the numerical difference in the two study groups.
Less than 10% of our study population had laboratory evidence of HIV. This is slightly less than that reported by Statistics South Africa in 2019, with an HIV prevalence of 13.5% (25). While Patel, M (1999) attributed this to a lack of association, this cannot be confirmed with our study (6).

Anaemia was the most common end organ damage due to plasma cell myeloma (77.16%), followed by renal insufficiency (35.65%). Hypercalcaemia was the least common, with only 20.06% of patients meeting the diagnostic criteria. This is in keeping with that described in the literature. Overall this was not very different to the USA although the cut-off references used are not exactly the diagnostic criteria used in our study as these have been updated (5). These findings were similar to a paper published by Chilli *et al* study from KwaZulu-Natal where anaemia, renal failure and hypercalcaemia were reported in 63.2%, 31.1% and 9.4% of patients respectively (17). The replacement of bone marrow by plasma cells results in anemia, which is strongly correlated with the degree of infiltration and the prognosis of the patient, as shown by Liu *et al* (26). The expression of chemokine CCL3 has been shown to be upregulated in PCM patients, further inhibiting erythropoiesis (26).
Our study showed that over 50% of patients had greater than 60% clonal plasma cells, as compared with Patel, M (1999) which showed 36.7% patients have a plasma cell percentage >30% and Kyle *et al* with 34% of patients with a plasma cell >60% (5,6). Comparing plasma cell percentages across studies is complicated by the fact that some investigators reported these on bone marrow aspirate while we chose to report on trephine biopsy, because clonality cannot be confirmed on bone marrow aspirates. Changes in the diagnostic criteria also make comparisons difficult, as reporting has subsequently changed. Bone marrow trephine biopsy findings are also very subjective and, considering that cases where the terms “sheets of plasma cells” and “diffusely infiltrated” were used and these were classified as ≥60%. A large proportion of patients (65.46%) did not meet the diagnostic criteria for flow cytometry / bone marrow aspirate. This was because flow cytometry is not routinely performed in many centers in South Africa as workup for PCM patients due to a heavier reliance on bone marrow trephine biopsies with immunohistochemistry as a cost-saving measure. This may also be explained by the fact that plasma cells due to their large size are lost during flow cytometry processing, differences in sampling and inclusion of survived plasma cells in

analysis (27). SFLC was not requested in a large proportion of our patient sample, as this test was not routinely available in the public sector in 2017-2019. This is a new addition to the updated diagnostic criteria and was just starting to gain popularity during our study period.

Our study showed that proportionately more patients presented with IgG as the monoclonal protein type followed by IgA isotype and then light chain disease. These findings are in keeping with the study by Patel, M (1999) and the review performed in the USA by Kyle *et al* (5,6). When looking at the serum and urine immunofixation findings in PLWH and HIV negative patients, their findings were similar to our patients in this study. Interestingly De Groot *et al* (2017) found all their patients to have an IgG subtype and suggested that PCM may have a relationship to the IgG response of HIV antigens (13). The study of Xiao *et al* (2022) was more in keeping with our findings, in that IgG, IgA and light chain subtypes were all represented in the PLWH (21).

This study highlights two additional important findings, first, the diagnosis of PCM in South Africa is not standardized, especially with regards to the use of flow cytometry and second, that there is limited access to healthcare for the diagnosis of PCM. Although flow cytometry is one of two options for proving plasma cell clonality (3), the Western Cape, Eastern Cape and Northern Cape do not routinely perform the procedure, due to financial implications and lack of requests from clinicians. Availability of flow cytometry across the country is also limited and, delay in transportation as well in processing of samples, impact on the survival of cells for laboratory analysis (27). Access to diagnostic tests in the smaller provinces is clearly limited given the smaller number of cases identified in these provinces. Further research is needed to assess the standardization of diagnostic criteria, as well as access to diagnostic procedures for PCM across South Africa.

This is a retrospective, pathology and laboratory based descriptive study across South African provinces without direct access to patient clinical history and records and there were therefore the following limitations to this study. Radiological investigations were available from the limited history captured from the bone marrow request forms. The radiological reports only mentioned the presence of lytic lesions without specifying the radiological investigations used and the number of lytic lesions found. Since a positive immunofixation result was used as the primary investigation to identify patients, all those who did not have an immunofixation performed, were not included in this study.

This therefore led to exclusion of patients with non-secretory PCM, patients having only a bone marrow aspirate and/or bone marrow trephine performed, those where a SFLC was used as the screening test as well as the ones diagnosed from a biopsy-proven plasmacytoma.
There are two methods used for electrophoresis, namely agarose gel electrophoresis and capillary zone electrophoresis, unfortunately the method of electrophoresis was not available. This and the subjectivity of interpretation of immunofixation are further limitation with this this study.

Patients who were diagnosed by a clinical hematologist or oncologist based on clinical grounds but who did not strictly fit the diagnostic criteria from a laboratory point of view, as well as patients for whom radiological investigations were used for diagnosis but not included in the history, were also not included.
Another limitation, regarding HIV status, was the large percentage of HIV unknown patients (26,18%) that may have either not consented to HIV testing or been screened using HIV rapid diagnostic tests which are not routinely captured on the LIS. We were unable to assess the racial demographics of all patients as race was only recorded for 50 out of the 718 (6.96%) patients.

Nevertheless, we were able to describe the demographics, laboratory investigations and diagnostic features of PCM patients diagnosed in the public sector from 2017-2019. We compared the demographics and diagnostic investigations across HIV status and found that PLWH were diagnosed at younger ages than HIV-negative patients, but that they had the same diagnostic criteria as HIV-negative patients and that there was no statistically significant difference in the laboratory tests.
This analysis provides insight into the demographic and disease characteristics of PCM patients across the public healthcare sectors in South Africa. Creating solid records of baseline patient disease characteristics using suggested diagnostic work-up and IMWG criteria provides a foundation for monitoring disease progression and response to treatment.

Funding: None.
Conflicts of interest: The authors declare that there is no conflict of interest.

REFERENCES

1. McKenna RW, Kyle RA, Kuehl WM, Harris NL, Coupland RW, Fend F. Plasma cell neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4t. Lyon: International Agency for Research on Cancer (lARC); 2017. p. 243–58.
2. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. Blood. 2009;113(22):5418–22.
3. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15(12):e538–48.
4. Waxman AJ, Mink PJ, Devesa SS, Anderson WF, Weiss BM, Kristinsson SY, et al. Racial disparities in incidence and outcome in multiple myeloma: A population-based study. Blood. 2010;116(25):5501–6.
5. Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003;78(1):21–33.
6. Patel M. An Epidemiological study of Multiple Myeloma in South Africa. University of the Witwatersrand, Johannesburg; 1999.
7. Oelofse D, Truter I. Incidence of haematological malignancies, Eastern Cape Province; South Africa, 2004–2013. Cancer Epidemiol. 2018;53:166–71.
8. Cowan AJ, Allen C, Barac A, Basaleem H, Bensenor I, Curado MP, et al. Global burden of multiple myeloma: A systematic analysis for the global burden of disease study 2016. JAMA Oncol. 2018;4(9):1221–7.
9. Cancer Association of South Africa. South African Statistics [Internet]. 2017 National Cancer Registry. [cited 2023 Aug 6]. Available from: https://cansa.org.za/south-african-cancer-statistics/
10. Cancer Association of South Africa. South African Statistics [Internet]. 2018 National Cancer Registry. [cited 2023 Aug 6]. Available from: https://cansa.org.za/south-african-cancer-statistics/
11. Cancer Association of South Africa. South African Statistics [Internet]. 2019 National Cancer Registry. [cited 2023 Aug 5]. Available from: https://cansa.org.za/south-african-cancer-statistics/
12. Fiorino AS, Atac B. Paraproteinemia, plasmacytoma, myeloma and HIV infection. Leukemia. 1997;11(12):2150–6.
13. De Groot JJB, Webb MJ, Raubenheimer JE, Struwig MC, Louw VJ. Concomitant HIV infection in

newly diagnosed multiple myeloma patients is hard to recognise and should be tested for routinely in areas of high endemicity. South African Med J. 2017;107(9):781–7.

14. Sengayi-Muchengeti M, Singh E, Chen WC, Bradshaw D, de Villiers CB, Newton R, et al. Thirteen cancers associated with HIV infection in a Black South African cancer patient population (1995-2016). Int J Cancer. 2023;152(2):183–94.
15. Munshi NC, Anderson KC, Bergsagel PL, Shaughnessy J, Palumbo A, Durie B, et al. Consensus recommendations for risk stratification in multiple myeloma: Report of the International Myeloma Workshop Consensus Panel 2. Blood. 2011;117(18):4696–700.
16. Rajkumar SV, Kumar S. Multiple Myeloma: Diagnosis and Treatment. Mayo Clin Proc [Internet]. 2016;91(1):101–19. Available from: http://dx.doi.org/10.1016/j.mayocp.2015.11.007
17. Chili LH, Mackraj I, Rapiti N. Profile and outcome of multiple myeloma with and without HIV treated at a tertiary hospital in KwaZulu-Natal, South Africa. PLoS One [Internet]. 2023;18(10 October):1–16. Available from: http://dx.doi.org/10.1371/journal.pone.0287304
18. Naidoo N, Erasmus RT, Grewal R, Zemlin AE. Electrophoresis test prevalence, requesting patterns, yield and related bone marrow biopsy findings at a South African tertiary hospital: A 5-year retrospective audit. South African Med J. 2017;107(3):270–3.
19. Govender K, Girdwood S, Letswalo D, Long L, Meyer-Rath G, Miot J. Primary healthcare seeking behaviour of low-income patients across the public and private health sectors in South Africa. BMC Public Health. 2021;21(1):1–10.
20. Jacob LA, Suresh Babu M, Lakshmaiah K, Babu KG, Lokanatha D, Rajeev L, et al. Multiple myeloma: Experience of an institute in limited resource setting. Indian J Cancer. 2017;54:340–2.
21. Xiao Z, Nguyen HL Le, Shao D, Murakhovskaya I. Impact of HIV Infection on Clinical Characteristics and Treatment Outcomes in African-American Patients with Multiple Myeloma: - a Retrospective Case-Control Study. Blood [Internet]. 2022;140(Supplement 1):5208–9. Available from: https://doi.org/10.1182/blood-2022-170334
22. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007;370(9581):59–67.
23. Bandera A, Colella E, Clerici M, Rizzardini G, Gori A. The contribution of immune activation and accelerated aging in multiple myeloma occurring in HIV-infected population. Aids. 2018;32(18):2841–6.
24. World Health Organization. Bridging the gap in South Africa [Internet]. Bulletin Volume 88.

2010 [cited 2020 May 12]. p. 797–876. Available from: https://www.who.int/bulletin/volumes/88/11/10-021110/en/

25. Statistics South Africa. Statistical Release P0302 [Internet]. Mid-year population estimates 2019. [cited 2023 Aug 6]. Available from: https://www.statssa.gov.za/publications/P0302/P03022019.pdf

26. Liu L, Yu Z, Cheng H, Mao X, Sui W, Deng S, et al. Multiple myeloma hinders erythropoiesis and causes anaemia owing to high levels of CCL3 in the bone marrow microenvironment. Sci Rep [Internet]. 2020;10(1):1–14. Available from: https://doi.org/10.1038/s41598-020-77450-y

27. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood [Internet]. 2008;111(8):3941–67. Available from: http://dx.doi.org/10.1182/blood-2007-11-120535

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

R14/49 Dr BT Wilding, et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200627

NAME:
(Principal Investigator) Dr BT Wilding, et al

DEPARTMENT: School of Pathology
Medical School
University

PROJECT TITLE: A retrospective study of the laboratory prevalence and epidemiological pattern of plasma cell myeloma in South Africa from 2017 to 2019

DATE CONSIDERED: 2020/06/26

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Professor J George; Drs T Wiggill & N Kone

APPROVED BY: B B Penny
Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2020/09/29

This clearance certificate is valid for 5 years from the date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore reports and re-certification will be due early in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

BTW
Principal Investigator Signature

02/10/20
Date