A comparison of 24-hour urine versus random urine samples for determination and quantification of Bence Jones protein in a South African population

By

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As the candidate's supervisor, I have approved this dissertation for submission.

Signed: DR V Gounden Name: DR Verena Gounden Date: 21/5/19

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(vii) My contribution to the project is as follows:
Literature review, research protocol development, ethical and hospital management approval, data collection and processing, statistical analysis, interpretation of data and journal article first draft write-up.
(viii) The contributions of others to the project are as follows:
1) Supervisor: Dr Verena Gounden: Formation of research idea and literature review, research protocol development, statistical analysis, interpretation of data and review of first draft write-up
2) Co-supervisor: Dr Nadine Rapiti: Research protocol review, data collection, review of manuscript

Dr Ashandree Reddy:  Date: 21/5/19

Dedication

Dedicated to my husband and family were strength, wisdom and comfort flow freely.

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Overview of the thesis

Introduction:

Multiple myeloma is a hematological cancer that has a high incidence of relapse despite intensive treatment regiments. The laboratory plays a vital role in treatment response evaluation. Measuring urine Bence Jones protein(BJP) is amongst the tests used to monitor response. The International Myeloma working group (IMWG) and College of American Pathologists recommend a 24-hour collection for BJP. Although a 24-hour urine collection is a definitive means to determine BJP excretion, it has several issues related to sample collection and is prone to inaccuracy. Protein to creatinine ratios have demonstrated good correlation with 24-hour urine. The aim of this study was to compare measured 24-hour urine collection to random urine for the quantitation of BJP in a South African population.

Method:

Known patients with multiple myeloma(MM) collected 24-hour urine as part of their routine clinical assessment for BJP, random urine samples were submitted following completion of the 24-hour collection. The measured 24-hour urine BJP was then compared to 2 estimated 24-hour BJP excretions. The estimated excretions were calculated as follows;

Estimation 1 (E1): Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) X10,

Estimation 2 (E2): Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) x 15mg/kg for women or x 20mg/kg for men.

All the 24-hour BJP results were classified according to IMWG treatment response criteria.

Results

When using the Wilcoxon paired test analysis, the measured 24-hour urine BJP was significantly different to both the E1 ($p=0.049$) and E2 ($p=0.049$) equations. But analysis following categorization of each patient per IMWG response criteria, indicated no significant difference in classification of treatment response using either the E1 or E2 estimation equations ($P=0.69$).

Conclusion:

24-hour urine collections are cumbersome. Random urine BJP estimates are simple, rapid and inexpensive. This study demonstrates that both the estimates of 24-hour BJP can be used to monitor response in patients with MM. This can be added to the body of evidence that random samples can be used to monitor patients' treatment response in MM.

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Part 1: The Review of Literature

Introduction

Multiple myeloma is a hematological cancer that develops in bone marrow and has a high incidence of relapse despite intensive treatment regiments. The laboratory plays a vital role in treatment response evaluation. Multiple myeloma has traditionally been based on the evaluation of serum and urine monoclonal protein concentrations via protein electrophoresis and immunofixation for response evaluation. The identification of paraprotein acts as surrogate for the tumour burden. [1]

We are continuously searching for reliable biomarkers in the hope that this could simplify and improve accuracy of medical decisions. This study reviewed the urine protein electrophoresis to determine if the measured 24-hour urine collection is comparable to random urine for the quantitation of Bence Jones protein (BJP) in a South African population. In doing so, we hoped to add to the body of existing knowledge and use random urine samples to monitor treatment response in patients known with multiple myeloma. A random urine sample may improve patient compliance to investigations, management and quality of life whilst on treatment.

The International Myeloma Working Group (IMWG) and College of American Pathologists recommend a 24-hour collection for Bence Jones proteins(BJP). [2-3] Although a 24-hour urine collection is a definitive means to determine BJP excretion, it has several issues related to sample collection and is prone to inaccuracy. [4-7] Protein to creatinine ratios have demonstrated good correlation with 24-hour urine. [4-6]

We intend with this literature review to examine the updated definition of multiple myeloma as well as review updated diagnostic criteria and treatment response criteria. We will also review literature that have performed similar studies in different populations.

Multiple Myeloma

Multiple myeloma is a hematological cancer that develops in bone marrow. It belongs to a group of diseases known as monoclonal gammopathies which are characterized by the neoplastic expansion of a single clone of plasma cells. Plasma cells are responsible for immunoglobulin production. The single clone malignant cell proliferation differs to the normal polyclonal distribution of plasma cells in a healthy individual. Monoclonal gammopathies produce increased amounts of a single clone of immunoglobulins that result in a disproportionate fraction of the number of plasma cells in the body. [2]

Examples of monoclonal gammopathies, also known as plasma dyscrasia's, include; multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma, solitary plasmacytomas, AL amyloidosis (light chain), Waldenstroms macroglobulinaemia and POEMS syndrome. Each of the monoclonal gammopathies have their own specific diagnostic criteria. These monoclonal gammopathies result from an abnormal overproduction of a single abnormal clone of a plasma cell or B lymphocyte resulting in increased production of either the intact immunoglobulin, free light chain component or both. The presence, level and type of the immunoglobulin have vital implications in diagnosis, staging and treatment of these diseases. [2]

Epidemiology

While age-standardized incidence rates differ with ethnicity from 3.9/100 000 in Chinese to 12.7/100 000 in African individuals, the data suggest that MM poses a significant worldwide health problem. [8] It consists of only 1% of all cancers but 10% of hematological malignancies in the United States. [9]
MM was responsible for 0.43% of newly diagnosed cases of malignancies in South Africa in 1999 with the incidence being reported at approximately 0.00054%. [10] While the incidence is highly variable among countries, studies indicate that the incidence of multiple myeloma has increased uniformly since 1990 with the largest increase in middle and low-middle income countries. [8,11]

The prevalence of multiple myeloma is higher in HIV positive compared to uninfected individuals. [12] This increases the disease burden of multiple myeloma in South Africa which has the largest HIV epidemic in the world having 7.1 million people living with HIV. [13] Multiple myeloma is also a disease that is more prevalent in the elderly with a median age of diagnosis being 65-70 years old. [8]

Pathophysiology and Diagnosis

The pathophysiology of multiple myeloma is complex. Briefly, the plasma cells, which originate from post follicular B cells, are characterized by complex chromosomal abnormalities. This results in dysregulation of oncogenes and suppressor genes. There is increasing evidence that suggests that the bone marrow microenvironment of tumor cells also perform a crucial role in the pathogenesis. The imbalances between receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin together with osteoclast activity factors significantly contribute to the development of myeloma bone disease. [15]

The pathophysiologic basis for the clinical sequelae of multiple myeloma comprises the skeletal, hematologic, renal, and nervous systems. Multiple myeloma is typically associated with four major dysfunctions i.e. Calcium, Renal, Anemia and Bone lesions, referred to by the acronym CRAB. Patients often have hypercalcemia due to bone lytic lesions caused by plasma cell tumours outgrowing their confined space in bone marrow and invading the bone. The breakdown of bone results in the release of calcium and hence, higher levels of serum calcium. The elevated serum calcium is defined as > 2.75mmol/L or > 0.25mmol/L of the upper reference limit. Patients can present with neurological features due to hypercalcaemia. Renal insufficiency results from deposition of free light chains in renal tubules. The monoclonal free light chains obstruct the renal tubules causing significant renal damage. The creatinine clearance <40 mL per min or serum creatinine >177 μmol/L is diagnostic of renal insufficiency. Anaemia is a consequence of overgrowth of plasma cells leading to crowding out of the cells that produce red blood cells. Erythropoietin production is also reduced in renal failure and contributes to the anaemia in patient with multiple myeloma. Anaemia in multiple myeloma is defined as a haemoglobin value of >2 g/dL below the lower limit of normal, or a haemoglobin value <10

g/dL. Bone lesions result in fractures often in the axial skeleton. The bone lytic lesions are caused by expansion of plasma cells in the bone marrow and is defined as one or more osteolytic lesions on skeletal radiography, computer tomography (CT), or positron emission tomography-CT. [2,15]

Understanding the natural progression of the multiple myeloma is important in order to appreciate the important role that the laboratory plays in the management of a patient with multiple myeloma. It is associated with significant mortality and morbidity and is considered largely incurable and fatal without treatment. With the introduction of new classes of effective drugs for the treatment of multiple myeloma, improved frequencies and degree of patient response has been observed. Many treatments have been shown to significantly prolong survival and simultaneously improve the quality of life for patients. Unfortunately, all patients will ultimately relapse after treatment and will require change to a more responsive therapy. This necessitates regular periodic monitoring of disease in order to detect relapse. Laboratory testing plays a vital role in monitoring response to treatment as well detecting relapse in patient on treatment. [1,2]

The diagnosis and monitoring of multiple myeloma can be accomplished by multiple means and typically includes a thorough clinical examination, history taking and laboratory testing. Laboratory tests include using immunoglobulin studies; serum and urine protein electrophoresis and immunofixation, serum free light chain(SFLC) analysis; bone marrow evaluation, full blood count with differential; serum chemistries: creatinine, calcium together with imaging. Imaging including conventional x-rays, CT, magnetic resonance imaging (MRI) and PET scans. [1,2]

Diagnosis of multiple myeloma is currently determined by the International Myeloma Working Group(IMWG). The IMWG has developed amongst others, guidelines for the diagnosis, management and response criteria for multiple myeloma. These criteria are constantly evolving and updated every few years to reflect our increasing knowledge of the disease. The most recent diagnostic criteria were published in 2014. The updated diagnostic criteria allows for treatment of patients who are at high risk of progression to symptomatic disease. Furthermore, these criteria may assist patients to potentially live longer if they were treated before serious organ damage occurred. [2,16]

The updated diagnostic criteria as per IMWG taken from the Lancet Oncology journal published in 2014 is as follows [2,16]:

Definition of multiple myeloma
Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma and any one or more of the following myeloma defining events:
• Myeloma defining events:
• Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
• Hypercalcaemia: serum calcium >0,25 mmol/L higher than the upper limit of normal or >2,75 mmol/L
• Renal insufficiency: creatinine clearance <40 mL per minor serum creatinine >177 μmol/L
• Anaemia: haemoglobin value of >2 g/dL below the lower limit of normal, or a haemoglobin value <10 g/dL
• Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT

• Any one or more of the following biomarkers of malignancy:
• Clonal bone marrow plasma cell percentage ≥60%
• Involved: uninvolved serum free light chain ratio ≥100
• >1 focal lesion on MRI studies

One of the new additions to the 2014 IMWG diagnostic criteria was the Myeloma Defining Events (MDE's) to the definition of myeloma. The MDE are associated with inevitable progression to end-organ damage and include: a) clonal bone marrow plasma cell percentage ≥60%. b) Involved: uninvolved serum free light chain ratio ≥100 and c) >1 focal lesion on MRI studies. This addition allows clinicians to start treatment earlier prior to end organ damage. [2,16]

Treatment response guideline

IMWG has guidelines for response to treatment as seen in Table 1. The uniform response criteria for multiple myeloma plays an essential role in disease management. It monitors for response and treatment relapse guides future therapy. The IWMG criteria include the measurement of serum free light chains, serum and urine electrophoresis and immunofixation, bone marrow analysis and imaging. [1,16]

Each laboratory investigation has its advantage and disadvantages in our setting and we have explored a few. Bone marrow examination directly identifies malignant cells, but the heterogeneous nature of the disease makes it problematic to use routinely to follow patients with multiple myeloma as it may not be representative and require repeated sample collection. Being an invasive procedure, bone marrow biopsies pose potential complications to the patient, such as bleeding and infection. They are also expensive and arduous to be performed at a regular interval for treatment monitoring. [17]

Light chains are more challenging to detected than complete immunoglobulins. [18] The serum free light-chain(SFLC) assay has increasingly been used, and in individual patients tracks well with proteinuria. [19-20] The greater sensitivity when compared to urine analysis, has brought forth the widespread use and incorporation of SFLC measurements into multiple guidelines for the management of myeloma, most recently as a myeloma defining event in asymptomatic patients. [1,21] However, the SFLC assay is not readily available in our province of KZN (referred to a NHLS laboratory in another province approximately 600km away). And due to inter-patient variation in the renal metabolism of light chains, quantification of proteinuria cannot be predicted by the SFLC concentration. [22-24] The IMWG states that once a diagnosis of multiple myeloma is made, a 24-hour urine protein electrophoresis(UPEP) and immunofixation should be done for patient monitoring and these measures are not replaceable with SFLC. [1,25]

It is presumed that due to the above-mentioned factors, urine and serum protein electrophoresis are commonly employed in our setting to monitor patients.

Our study focused on urinary monoclonal free light chains as it is part of the response criteria in multiple myeloma and it is measured in the Chemistry department at the National Health Laboratory Service, Inkosi Albert Luthuli Central Hospital. It is an available laboratory test for our population and fairly simple to perform.

Monoclonal free light chains

Monoclonal free light chains (FLCs) appearing in urine, are referred to as Bence Jones proteins (BJP). This was first described by Dr. Henry Bence Jones over 150 years ago. Detection and measurement of BJPs are utilized to aid in the diagnosis and monitoring of monoclonal gammopathies. [2,26] Once renal tubular reabsorption is saturated; BJP is present in urine. In approximately 20% of MM cases, BJP may occur in the absence of a monoclonal band in the serum thus making it a valuable test in the detection of this malignancy. [26, 27]

BJP may be quantified by means of urine protein electrophoresis. This method involves separation of charged proteins in a liquid medium under the influence of an electrical field. Following electrophoresis of the urine specimen and staining of the gel, the size of the BJP peak is measured using densitometry scan of the peak. The percentage area of the peak is then multiplied by the total urine protein concentration of the sample to provide a semi- quantitative value for the BJP. Confirmation of the presence of BJP following urine protein electrophoresis is performed via urine immunofixation electrophoresis(UIFE). The principle of UIFE involves applying antisera to the separated proteins on the gel. Antigen-antibody complexes precipitate and are trapped within the gel matrix. The complexes are then stained and visualized. [18]

Response criteria

The Bence Jones protein quantitated for as a 24-hour urine is used to monitor treatment response in categories described by the IMWG guidelines. The article; International myeloma working group consensus criteria for response and minimal residual disease assessment in multiple myeloma printed in the Lancet Oncology journal in 2016 is presented below, see Table 1. [1,16]

Stringent complete response	Complete response as defined below plus normal FLC ratio and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial remission (VGPR)	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response (PR)	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD) of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD) of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease (poor response)	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0·5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease
Clinical relapse	Clinical relapse requires one or more of the following criteria: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); Definite increase in the size of existing plasmacytomas or bone lesions. A

	definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD of the measurable lesion; Hypercalcaemia (>11 mg/dL); Decrease in haemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; Rise in serum creatinine by **2 mg/dL** or more from the start of the therapy and attributable to myeloma; Hyperviscosity related to serum paraprotein
Relapse from complete response (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% plasma cells in the bone marrow; Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcaemia see above)
Relapse from MRD negative (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% clonal plasma cells in the bone marrow; Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcaemia)

Table 1. International myeloma working group consensus criteria for response and minimal residual disease assessment in multiple myeloma
SPD=sum of the products of the maximal perpendicular diameters of measured lesions.
CRAB features=calcium elevation, renal failure, anaemia, lytic bone lesions.

Traditional serum and urine assessment of monoclonal proteins and bone marrow assessment was used as response criteria in multiple myeloma. Recent efforts have focused on the identification of residual tumour cells in the bone marrow by means of flow cytometry or gene sequencing. In addition, sensitive imaging techniques can be used to detect the presence of residual disease beyond the bone marrow. Combining these different methods, the International Myeloma Working Group has defined new response categories of minimal residual disease. This permits consistent reporting inside and outside of clinical trials. [1,16]

BJP can be used to differentiate the treatment response categories and guide therapy. The International Myeloma Working Group (IMWG) and the College of American pathology recommend a 24-hour urine collection for quantification of urine BJP. [2,3] The advantage of the 24-hour urine collection is that it defines BJP excretion over the entire 24-hours and directly relates to published data on 24-hour BJP excretion. But it has several issues especially those related to sample collection and is often subject to error and may result in inaccurate BJP quantitation. [4-7]

In particular, the impracticality of a 24-hour collection together with high likelihood of incomplete collections hinder the accuracy of the test. Other disadvantages for the patient include the inconvenience of storage and transport of the samples to the clinic as these are most often in large 5-liter plastic containers. Many of these patients carry their 24-hour urine collections, travelling several hundred kilometers using public transport to reach the haematology clinic. This is not ideal for maintaining sample stability as well as inconvenient and embarrassing for the patient**.** [28]

It is also more time consuming for the laboratory to supervise as the completed 24-hour urine samples must be accurately weighed for the correct volume. An aliquot of the sample together with the recorded 24-hour volume is sent to the referral laboratory for analysis. Some aliquots are sent without volumes and these samples are unfortunately rejected as the calculation for BJP requires a volume. In these instances, no result can be provided to the clinician for patient management in regard to the recognized response criteria. An important factor affecting 24-hour collections is that total urinary protein has a variation with urine volume and hence, can be variable depending on patients' fluid status or medications (e.g. diuretics). [5,29-30]
Random urine collections on the other hand are easy to obtain. There is rapid transfer to laboratory which avoids potential degradation of protein. It can also be collected at any time of day and there is no need for the patient to store the sample and transport it to the laboratory.

Hence the use of random or early morning urine collections have been suggested to avoid the problems associated with 24-hour collections. The main concern with random urine is that the synthesis and release of BJP may be variable throughout the day. But, the clinical utility of urine protein results is improved when expressed as a ratio to urine creatinine. [4-6] As creatinine excretion in urine is fairly constant throughout the 24-hour period, measurement of protein creatinine ratios (PCR) allows correction for variations in urine concentration. The use of PCRs has become widespread for routine urine protein analysis and several studies have demonstrated good correlation with the 24-hour collection. [4-6]

Similar studies

Whilst the use of Bence Jones protein creatinine ratios has emerged as an alternative to the 24-hour collection, there are few studies that have examined its correlation with the 24-hour collection and no reported studies to our knowledge reviewing its utility in an African population**.** [29-30]

A previous study demonstrated that it may be possible to use the protein/creatinine ratio from random urine samples to estimate the 24-hour BJP excretion. [29] Another study concluded that protein concentrations in the same individual are relatively constant. This group also demonstrated that early morning spot specimens had a linear relation with measured 24-hour BJP collections and were preferred over the random urine collection. [30].

Conclusion

The need for simple, easily available test for treatment response in multiple myeloma in our population can assist and improve patient manage. We decided to compare the use of measured 24-hour urine collection to random urine for the quantitation of BJP in a South African population with this mind. Very few similar studies have been done in different population groups and have shown some comparability.

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Part 2: A submission ready manuscript.

CHAPTER 2

A comparison of 24-hour urine versus random urine samples for determination and quantification of Bence Jones protein in a South African population

Prepared according to the Instructions for Authors of Clinical Biochemistry

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Abstract

Objectives:

The International Myeloma Working Group (IMWG) and College of American Pathologists recommend a 24-hour collection for Bence Jones proteins(BJP). Although a 24-hour urine collection is a definitive means to determine BJP excretion, it has several issues related to sample collection and is prone to inaccuracy. Protein to creatinine ratios have demonstrated good correlation with 24-hour urine. The aim of this study was to compare measured 24-hour urine to random urine collections for the quantitation of BJP in a South African population.

Method:

Known patients with multiple myeloma(MM) collected 24-hour urine as part of their routine clinical assessment for BJP, random urine samples were submitted following completion of the 24-hour collection. The measured 24-hour urine BJP was then compared to 2 estimated 24-hour BJP excretions which were calculated as follows; Estimation 1 (E1): Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) × 10, Estimation 2 (E2): Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) × 15mg/kg for women or × 20mg/kg for men. All the 24-hour BJP results were classified according to IMWG treatment response criteria.

Results:

When using the Wilcoxon paired test analysis, the measured 24-hour urine BJP was significantly different to both the E1 (p=0.049) and E2 (p=0.049) equations. But analysis following categorization of each patient per IMWG BJP response criteria, indicated no significant difference in classification of treatment response using either the E1 or E2 estimation equations (P=0.69).

Conclusion:

24-hour urine collections are cumbersome. Random urine BJP estimates are simple, rapid and inexpensive. This study demonstrates that both the estimates of 24-hour BJP can be used to monitor response in patients with MM. This can be added to the body of evidence that random samples can be used to monitor patients' treatment response in MM.

1. Introduction

Plasma dyscrasias (PD) are a group of disorders, which includes multiple myeloma, where a clone of plasma cells or B-lymphocytes have the ability to secrete a homogenous immunoglobulin (Ig) or its components. These may be identified as a monoclonal peak on analysis by serum or urine protein electrophoresis [1]. The presence, level and type of monoclonal Ig have important implications in diagnosis, staging and treatment of disease states [2].

The core diagnostic features of multiple myeloma (MM) include the presence of neoplastic plasma cells on bone marrow aspirate, radiological evidence of osteolytic lesions and detection of monoclonal Igs in serum or urine [1]. It is the second most common haematological cancer accounting for 1% of all malignancies worldwide. MM was responsible for 0.43% of newly diagnosed cases of malignancies in South Africa in 1999 with the incidence being reported at approximately 0.00054%. [3] While the incidence is highly variable among countries, studies indicate that the incidence of MM has increased uniformly since 1990 with the largest increase in middle and low-middle income countries. [4,5] The prevalence of MM is higher in HIV positive compared to uninfected individuals. [6] This increases the disease burden of MM in South Africa which has the largest HIV epidemic in the world having 7.1 million people living with HIV. [7]

Monoclonal free light chains (FLCs) appearing in urine, are referred to as Bence Jones proteins (BJP). This was first described by Dr. Henry Bence Jones over 150 years ago. Detection and measurement of BJPs are utilized to aid in the diagnosis and monitoring of monoclonal gammopathies. [8,9] Once renal tubular reabsorption is saturated; BJP is present in urine. In approximately 20% of MM cases, BJP may occur in the absence of a monoclonal band in the serum thus making it a valuable test in the detection of this malignancy. [8, 10]

BJP may be quantified by means of urine protein electrophoresis. Following electrophoresis of the urine specimen and staining of the gel, the size of the BJP peak is measured using densitometry scan of the peak. The percentage area of the peak is then multiplied by the total urine protein concentration of the sample to provide a semi- quantitative value for the BJP. Confirmation of the presence of BJP following urine protein electrophoresis is performed via immunofixation.

The International Myeloma Working Group (IMWG) and the College of American pathology recommend a 24-hour urine collection for quantification of urine BJP. [9, 11] Although a 24-hour urine is a definitive means to determine renal protein excretion, it has several issues especially those related to sample collection (as summarized in Table 1). In particular, the impracticality of a 24-hour collection together with high likelihood of incomplete collections hinder the accuracy of the test. Hence the use of random or early morning urine collections have been suggested to avoid the problems associated with 24-hour collections. The clinical utility of measured urine protein is improved when expressed as a ratio to urine creatinine. [12-14] As creatinine excretion in urine is fairly constant throughout the 24-hour period, measurement of protein creatinine ratios (PCR) allows correction for variations in urine concentration. The use of PCRs has become widespread for routine urine protein analysis and several studies have demonstrated good correlation with the 24-hour collection. [11-13]

Whilst the use of Bence Jones protein creatinine ratios has emerged as an alternative to the 24-hour collection, there are few studies that have examined its correlation with the 24-hour collection and no reported studies to the authors knowledge reviewing its utility in an African population**.** [14-17]

	Advantages	**Disadvantages**
Random **Urine**	• Easy to obtain • Rapid transfer to laboratory which avoids potential degradation • Collected any time of day • No need for patient to store sample and transport to laboratory	• Synthesis and release of BJP may be variable throughout the day
24-hour **Urine**	• Defines BJP excretion over the entire 24-hours • Directly relates to published data on 24-hour BJP excretion	• Inconvenient/complex for patient-collection, storage, transport to hospital. • Frequently incomplete collections • More expensive for laboratory to supervise • Total urinary protein has a variation with urine volume

Table 1 Comparison of Random and 24-hour urine collection for BJP [12-15]

The haematology clinic at King Edward VIII Hospital (KEH) is the referral center for the entire province of Kwa-Zulu Natal (KZN), South Africa, for the management of patients with multiple myeloma and other plasma cell dyscrasias. Many of these patients carry their 24-hour collections, travelling several hundred kilometers using public transport to reach the haematology clinic. This is not ideal for maintaining sample stability as well as inconvenient and embarrassing for the patient**.** [18]

MM is largely incurable and despite new therapy options, most patients relapse and require change in management. Laboratory testing plays a vital role in monitoring response to treatment as well detecting relapse in patient on treatment. [9] This, together with the previously described issues related to 24-hour urine collections prompted us to examine the utility and validity of measured 24-hour urine compared to random urine collections for the quantitation of BJP in a South African population.

2. Material and Methods

Study participants were recruited based on a known diagnosis of PDs from the Haematology clinic at King Edward VIII Hospital, Durban. Samples were collected over a period of 2 years (2016-2018). All participants had the diagnosis of multiple myeloma (per IMWG criteria) and were at different stages of disease and treatment.

Each participant collected a 24-hour urine sample for BJP following a standard protocol as part of the routine clinical assessment. The 24-hour collection was started the day before the clinic visit. On submission of the 24-hour collection, the participants immediately collected a random urine sample as per instructions provided. Thymol was used as the preservative for the 24-hour urine sample and no preservative was utilized for the random sample. Both samples were submitted to the laboratory immediately. The 24-hour collections were analysed as per routine by the chemical pathology laboratory.

The random urine samples were analysed for urine total protein and creatinine. Aliquots of the random urine samples were then frozen at -70°C and stored for a maximum of one month (stability as per manufacturer) until the urine protein electrophoresis (UPEP) was performed. [18]

For both random and 24-hour urine collections, UPEP was performed using the Sebia Hydragel 7 HR kit run on the Sebia Hydrasys (Sebia, Norcross, GA, USA). Quantitation of UPEP fractions was performed using the Sebia Hydrasys densitometer system and Phoresis software. Acid violet staining was used and the sensitivity of this method allows BJP to be detected at concentrations of 15-20 mg/L of the original urine. Urine samples for immunofixation electrophoresis (IFE) analysis were concentrated using BJP concentrators from the Sebia Hydrasys kit for all urine total protein samples measuring < 0.7g/L. [18] Urine total protein(UTP) and urine creatinine were measured using standard spectrophotometric methods on the Siemens Advia 1800 chemistry analyser (Siemens Diagnostics, Tarrytown, NY, USA). A dye binding method using pyrogallol red was used to quantify UTP. [19] Urine creatinine was measured using the modified kinetic Jaffe method. [20]

Only those 24-hour urine sample that were positive for BJP, had their respective random samples analysed to determine comparability. The measured 24–hour BJP excretion was calculated as follows: %BJP peak on densitometer × UTP (g/L) × 24-hour urine volume (L) and multiplied by 1000 for mg/24hr. The estimated 24 BJP using the random urine values were calculated as per the two different formulae below:

Estimation 1 (E1):

Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) ×10

Note: For estimation 1 (E1), a factor of 10 was utilized because while daily excretion of creatinine is dependent on muscle mass, an average daily loss of 10mmol of creatinine can be expected. [13]

Estimation 2 (E2):

Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) × 15mg/kg for women or × 20mg/kg for men (convert mg/kg to mmol/kg by multiplying 0.00884).

Note: The second method includes an estimation of daily creatinine excretion based on body mass (in kilograms). [16]

The estimated 24-hour BJP values were then used to classify patients according to their treatment response based on IMWG criteria. Very good partial response (VGPR) signified a concentration of <100mg/24hr, partial response 100-199mg/24hr and progressive disease was all those patients with ≥ 200mg/24hr. [21]

Demographic details and clinical histories were collected from the patient's clinical records.

The Body mass index (BMI) was calculated as weight/height^2 (kg/m^2) and categorized according to WHO. Statistical analyses were performed using Microsoft® Excel (Microsoft® Office 2016, Microsoft, USA) and MedCalc for Windows, version 10.0 (MedCalc Software, Ostend, Belgium). The Shapiro-Wilk test was used to assess normality. For non-parametric data Spearman rank correlation and Passing Bablock regression analysis was utilized for comparison of different estimated 24-hour BJP equations to the measured 24-hour BJP. Categorical data was compared using the Kruskal Wallis test. Wilcoxon paired sample analysis was used to compare continuous variables. A p value of <0.05 was deemed to be statistically significant.
Ethical approval to conduct the study was acquired from the Biomedical Research Ethics Committee (BREC), University of Kwazulu-Natal (ref. no. BE509/15). Written informed consent was taken from each participant in English or isiZulu depending on their requirement.

3. Results

A total of 66 paired 24-hour and random urine samples were collected. Of these, 22 had detectable BJP on 24-hour UPEP and 19 had a quantifiable BJP in g/24hrs. Three patients had faint bands below detectable limit (< 15mg/L) on the measured 24-hour urine with % BJP calculated but, did have a quantifiable BJP peak on their paired random urine sample. The urine TP for those 3 24-hour samples were 0.1g/L, 0.07g/L and 0.05g/L which was much lower than their random paired samples 1.6g/L, 1.1g/L and 1.8g/L. This could account for the non-quantifiable bands in the measured 24-hour urine samples. One sample had a quantifiable 24-hour BJP peak (TP 0.1g/L) with no peak on the random urine sample (TP 4.2g/L) but the monoclonal band was present on UIFE.

The 22 samples were from 19 patients as 3 patients had repeat collections within the study period. There were 10 females and 9 males with 18 of the 19 patients being black African. The remaining one patient was of Indian descent. Table 2 presents the 19 patients with their demographics, immunotyping and other relevant results. Of note, there is only a record of 10 patients tested for HIV, with only one being positive. Serum Free Light Chain's (SFLC) were also only measured in 7 of these patients. Of the 19 patients, 2 did not have UIFE analysis performed despite having detectable BJP on UPEP and the UIFE being suggested by the

reporting pathologist. The mean age was 55,8 years old (SD ±6,6)) and the mean BMI was 27,5 m^2/kg ((SD ±5,4). Refer to table 3.

No	Age	Gender	Race	SIFE	UIFE	Total Serum Calcium	Albumin	eGFR	TP	HB	HIV status	SFLC ratio
1	58	F	B	IgA K and free K	Free K	2,65	42	17	81	5,2	Neg	Nil
2	74	M	B	IgG K and free K	n/a	2,03	22	15	98	5,9	Nil	Nil
3	57	F	B	IgG K and free K	Free K	1,89	17	>60	41	5,7	Nil	Nil
4	61	M	B	IgG K and free K	Free K	2,06	16	>60	108	7,7	Nil	Nil
5	59	F	B	IgG K and free K	Free K	2,2	26	24	82	9,3	Nil	Nil
6	54	F	B	IgA K and free K	IgA K and free K	1,7	20	7	86	7,7	Neg	Nil
7	59	M	B	IgA K	Free K	2,17	29	36	95	8,6	Neg	4.73
8	58	M	B	IgG K	IgG K	3,18	20	18	153	6,4	Neg	Nil
9	63	M	B	IgG K	n/a	2,65	28	37	100	8,8	Neg	Nil
10	47	M	B	Free L	Free L	3,36	34	16	73	5,9	Pos	Nil
11	56	F	B	Free L	Free L	2,6	40	48	73	13,3	Nil	0,01
12	53	F	B	IgG L and free L	IgG L and free L	2,2	35	19	83	9,1	Neg	Nil
13	65	M	B	Free K	Free K	2,28	40	>60	67	7,9	Nil	1,76
14	57	F	I	IgG K	IgG K and free K	1,9	19	9	96	7,4	Nil	Nil
15	47	F	B	IgA K	IgA K and free K	3,49	16	8	140	6,7	Neg	102,23
16	49	M	B	IgA K	IgA K and free K	2,04	21	50	109	9,2	Neg	0,76
17	49	F	B	IgA L	Free L	2,6	21	23	118	6	Nil	Nil
18	55	M	B	Free L	Free L	2,19	38	>60	65	10,3	Nil	0,01
19	56	F	B	Free K	Free K	2,59	37	10	72	6,6	Neg	leaked
Mean (SD)						2,4(0,5)	27,4(8,9)	10,0	91,6(25,9)	7,8(1,9)		

Table 2 Patient characteristics

Notes: Age in years. SIFE; Serum immunofixation electrophoresis, UIFE; Urine immunofixation electrophoresis, M; male, F; female, B; black, I; Indian, K; Kappa, L; Lambda, TP; Total protein, HB; Hemoglobin, HIV; Human Immunodeficiency Virus, Neg; negative, Pos; positive, SFLC; Serum Free Light Chain. eGFR; estimated Glomerular Filtration Rate in ml/min/1.73$m^{2.}$
Reference ranges: SFLC; 0.26-1.65, Calcium 2.15 - 2.55mmol/L, Albumin 35-52g/L.

Parameters	Mean (±SD) Median(range)
Total number of samples	N=22
Age (years)	55.8 (±6.6)
BMI (m^2/kg)	27.5(±5.4)
Measured 24-hour BJP (mg/24hr)	2480 (250-14400)
E1 (mg/24hr)	1256 (211-7143)
E2 (mg/24hr)	1403 (267-9597)

Table 3 Summary data of patient characteristics

[note E1 refers to (E1) Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) ×10 and E2 refers to Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) × 15mg/kg for women or × 20mg/kg for men (convert mg/kg to mmol/kg by multiplying 0.00884).

Using Wilcoxon paired test analysis, the measured 24-hour urine BJP was significantly different to both the E1 (p=0.049) and for the E2 (p=0.049) equations. Spearman rank correlation for both estimation equations E1 and E2 was 0.893 when compared to the measured 24-hour BJP. On Kruskal Wallis analysis following categorization of each patient per IMWG BJP response criteria, there was no significant difference in classification of treatment response using either the E1 or E2 estimation equations when compared to the measured 24-hour urine BJP results (P=0.69). Results of Passing Bablock regression analysis are shown in Figures 1 and 2. The E2 estimation equation shows a smaller proportional bias with a slope of 0.968 as compared to the E1 estimation equation slope of 0.671 when compared to the measured 24-hour BJP

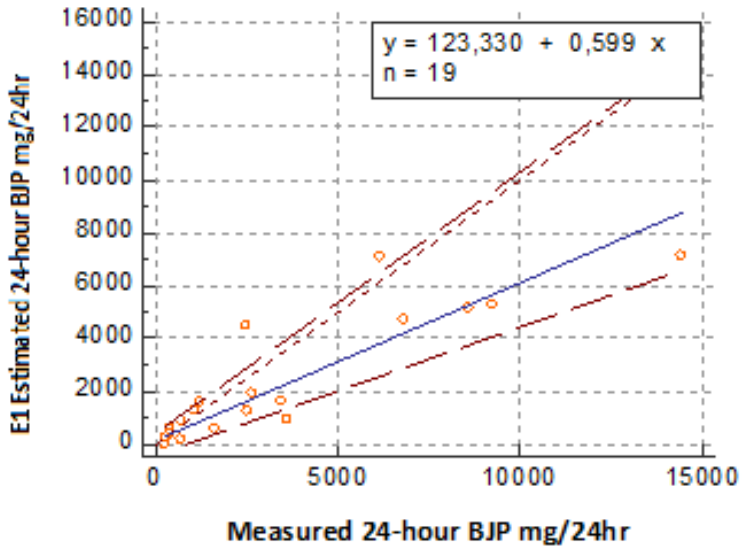

Figure 1

Regression analysis of Measured 24–hour BJP excretion versus E1 estimation for 24 hour BJP

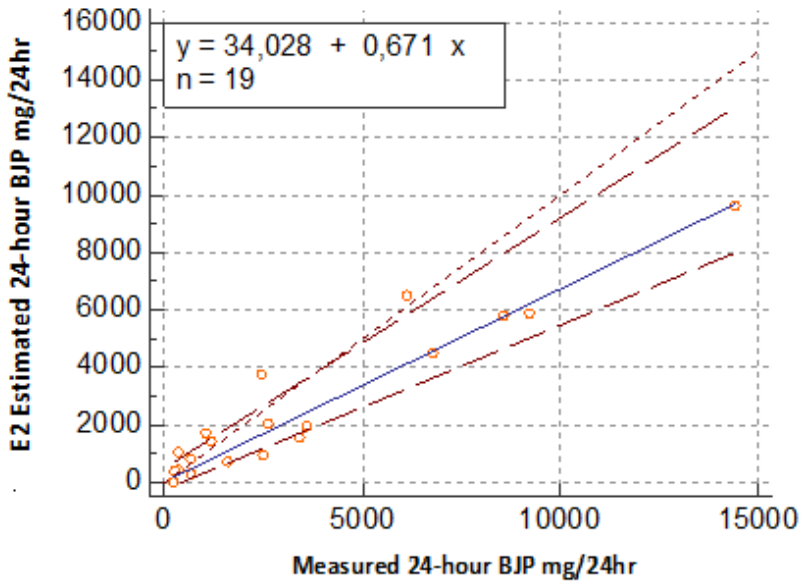

Figure 2

Regression analysis of Measure 24–hour BJP excretion versus E2 estimation for 24-hour BJP

Key for figure 1 and figure 2

—— : Regression line

– – – : 95% Confidence intervals

o : Sample

4. Discussion

The average age of patients in this study was 55,8 years old, which is much younger when compared to the western countries with the average age at diagnosis ranging from 65-70 years. [22] This could be related to the high incidence of HIV in our population. HIV infection is more prevalent among younger than older patients hence, HIV-positive MM patients present at a significantly lower age. [6,22] Ethnicity has been found to effect incidence of MM. [23] Our data showed only 1 HIV positive patient from the 10 patients tested making ethnic disparities an alternative reason for the lower age.

The E2 estimation demonstrations a closer correlation and smaller proportional bias with the measured 24-hour BJP compared to E1 estimation equation. Our study revealed that both methods used to estimate 24-hour BJP were not comparable to the actual measured 24-hour BJP however when the estimated BJPs were used to classify patients according to IMWG treatment response, there was no significant difference with the performance of the measured 24-hour BJP and the estimated BJP using the E1 and E2 equations. This is key with regards to being able to use the random specimens for monitoring of disease. This study indicates that both the estimates of 24-hour BJP can be used to monitor response in patients with MM. This is in keeping with prior findings in other studies performed in different population groups. (16,17)

A previous study demonstrated that it may be possible to use the protein/creatinine ratio from random urine samples to estimate the 24-hour BJP excretion. [16] Another study concluded that protein concentrations in the same individual are relatively constant. This group also demonstrated that early morning spot specimens had a linear relation with measured 24-hour BJP collections and were preferred over the random urine collection. [17]. Because patients travelled long distances and arrived at the Haematology clinic at varying times, early morning specimens where a challenge to collect. Despite this, our study was still able to demonstrate that a random sample can be used to determine an estimate of the measured 24-hour BJP and can be used to monitor disease response to treatment.

As a result of only including patients with densitometrically quantifiable BJP on the measured 24-hour BJP the small sample size was a limitation, however this was also a limitation noted in other studies reviewing use of random urines for BJP estimation. (16,17) Measuring the creatinine on the 24-hour urine collections to verify the accuracy of collection would have been beneficial. [17] Another limitation is the challenges associated with the method to quantify BJP. Different proteins have varying affinities for the dyes used to stain electrophoretic gels, and thus a lack of linearity of the densitometry response may be seen. BJP may also co-migrate with other proteins or present with several bands making it complex to define the BJP peak correctly by densitometry. The measurement of BJP is not standardized and in order to minimize the mentioned analytical variability, it is suggested that patients should be followed up at the same laboratory, which was adhered to in this study. [24] We suggest using the random BJP to monitor

known patients with MM who already have confirmed BJP on immunofixation to minimize the above-mentioned limitations associated with measuring BJP's on electrophoresis.

Light chains are more challenging to detected than complete immunoglobulins. [25] The serum free light-chain(SFLC) assay has increasingly been used and tracks well with proteinuria in individual patients. [26,27] The greater sensitivity when compared to urine analysis, has brought forth the widespread use and incorporation of SFLC measurements into multiple guidelines for the management of myeloma, most recently as a myeloma defining event in asymptomatic patients. [9,28]. All the study participants had a SPEP and UPEP but surprisingly only a few had SFLC's. We found only 7 patients had SFLC's and 1 of the 7 samples had leaked during transit. The SFLC assay is not readily available in our province of KZN. And due to inter-patient variation in the renal metabolism of light chains, quantification of proteinuria cannot be predicted by the SFLC concentration. [29-31] 15 of the 19 patients had GFR's < 60ml/min/1.73m^2 which may affect the renal metabolism of SFLC's. The IMWG states that once a diagnosis of MM is made, a 24-hour UPEP and immunofixation should be done for patient monitoring and these measures are not replaceable with SFLC. [21,31]
MM is associated with significant mortality and morbidity and is considered largely incurable and fatal without treatment. With the introduction of new classes of effective drugs for the treatment of multiple myeloma, improved frequencies and degree of patient response has been observed. Many treatments have been shown to significantly prolong survival and simultaneously improve the quality of life. Unfortunately, all patients will ultimately relapse after treatment and will require change to a more responsive therapy. This necessitates regular periodic monitoring of disease in order to detect relapse. Laboratory testing plays a vital role in monitoring response to treatment as well detecting relapse in patient on treatment. [9,21]

This study is the first to use and to demonstrate the utility of the estimate 24-hour urine BJP in an African population group. Both the E1 and E2 calculations are simple to perform and UTP and urine creatinine measurements are easily available on routine chemistry analysers.
This, together with other studies can be used by IMWG to add to their body of evidence for future use of estimated 24-hour BJP for patient monitoring. [16,17]

5. Conclusion

The random urine BJP estimates are simple, rapid, easily available and an inexpensive method for monitoring known patients with MM including light-chain disease. We have demonstrated that when using the IMWG response classification, the estimating equations, E1 and E2, did not differ from the measured 24-hour BJP. Further studies with larger cohorts can be conducted to validate the constant protein to creatinine ratios in random urine samples throughout the 24-hour period in patients with BJP to verify the accurateness of using a random sample.

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Conflict of interest:
The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions:
1) Verena Gounden: Formation of research idea and literature review, research protocol development, statistical analysis, interpretation of data and review of first draft write-up
2) Ashandree Reddy: Literature review, research protocol development, ethical and hospital management approval, data collection and processing, statistical analysis, interpretation of data and journal article first draft write-up.
3) Nadine Rapiti: Research protocol review, data collection, review of manuscript

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Appendices

Appendix 1: The Study Protocol

Research Protocol: MMed

Pilot study: A comparison of 24-hour urine versus random urine samples for determination and quantification of Bence Jones protein

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February 2016

Title of study

A comparison of 24-hour urine versus random urine samples for determination and quantification of Bence Jones protein.

Aims

1 To compare accuracy of random urine Bence Jones:creatinine ratios (mg/mmol) to the gold standard 24-hour Bence Jones protein quantitation (mg/24 hrs)

Secondary objectives

1. To assess the accuracy of currently available equations for estimation of 24-hour BJP quantitation from random urine BJP: creatinine ratios
2. To develop an equation for estimation of 24-hour BJP values using random urine BJP:creatinine ratio

Background and literature

Plasma dyscrasias are a group of disorders associated with the presence of a monoclonal band (M protein) from malignant or nonproliferative population of cells. Examples of plasma dyscrasias include multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS) and plasmacytoma as well as conditions such as amyloidosis and Waldenstroms macroglobulinaemia. [2] These monoclonal gammopathies result from an abnormal overproduction of a single abnormal clone of a plasma cell or B lymphocyte resulting in increased production of one immunoglobulin type (either the intact immunoglobulin, free light chain component or both).[1]The presence, level and type of the immunoglobulin have vital implications in diagnosis, staging and treatment of these diseases. [8]This study focuses on MM as it is the second most common cancer of blood and accounts for 1% of all malignancies worldwide.[6] It is also associated with significant mortality and morbidity and is considered largely incurable.MM accounted for 0.43% of newly diagnosed cases of malignancies in South Africa in 1999 which makes the incidence approximately 0.00054% in our population of 47.8 million.[7]

The monoclonal immunoglobulin (M protein) is recognized as a band of restricted migration on serum protein electrophoresis (SPEP) or urine protein electrophoresis (UPEP).[1] Bence Jones protein (BJP) refers to the presence of a band of restricted migration on SPEP or UPEP representing a free light chain that is utilised to identify, diagnose and monitor patients with plasma cell dyscrasias in particular multiple myeloma. BJP is also important for the diagnosis of light chain myeloma as it is the only indicator of response to therapy.

The international myeloma working group (IMWG) recommendations provide essential procedures for the diagnosis and follow up of patients with MM. The investigations of interest to this study include SPEP and immunofixation as well as 24-hour urine collection for proteinuria,

electrophoresis and immunofixation. The Bence Jones protein quantitated for as a 24-hour urine is used to monitor treatment response (see Table1) in categories described by the IMWG guidelines.

Near complete remission	Paraprotein visible by IFE but not on electrophoresis of serum or urine samples
Very good partial remission (VGPR)	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥ 90% reduction in serum M-protein plus urine M-protein level < 100 mg/24 h or ≥ 50% reduction of serum M-protein and reduction in 24-hours urinary M-protein by≥90% or to < 200 mg/24 hr.
Partial response (PR)	If serum and urine M-protein are not measurable, and serum free light chain assay is also not measurable , ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥ 30%
Progressive disease (poor response)	24-hour urine Bence Jones protein :25% increase from nadir of urine M-component (the absolute increase must be ≥200 mg/24 h)
Relapse from complete response	Reappearance of serum or urine M-protein on electrophoresis or immunofixation.

Table 1. IWMG Treatment response categories that include BJP.

Whilst the performance of urine electrophoresis and BJP quantitation is a relatively simple analytical procedure, the collection of urine over a 24-hour period is often subject to error and may result in inaccurate BJP quantitation. The preanalytical errors that can lead to a less reliable result include collection beyond or under the 24hour period is common as well as contamination and improper storage of urine during collection. The process is inconvenient and time consuming for patients and results in delay of sample analysis and ultimately treatment. The 24-hour urine sample is also more expensive for the laboratory to supervise.[4]On the other hand, random urine collections are easy to obtain, allow rapid transfer to the laboratory and may avoid potential contamination and degradation of protein. A random specimen is a non-timed specimen that may be taken at any time of the day. The use of random urine protein/albumin: creatinine ratios have been shown to be equivalent to 24-hour collection for the measurement of urine protein and albumin, hence this study employed a protein(BJP): creatinine ratio.

Key references

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7. Visser HF et al. Retrospective review of multiple myeloma and immunosecretory disorder cases diagnosed in a tertiary setting. SA orthopaedic journal.Summer 2008: 38-43

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Study design

This is a pilot study as literature on this topic is scarce and previous studies involved very small sample numbers. It is a quantitative observational study

The validity of the study lies in a random urine sample being compared to the gold standard 24-hour urine collection for Bence Jones Protein.

Study population and location

Known patients with plasma dyscrasias enrolled at the Haematology clinic at King Edward VIII Hospital, Durban for which 24-hour urine collections will be performed for Bence Jones protein analysis as part of routine clinical assessment.

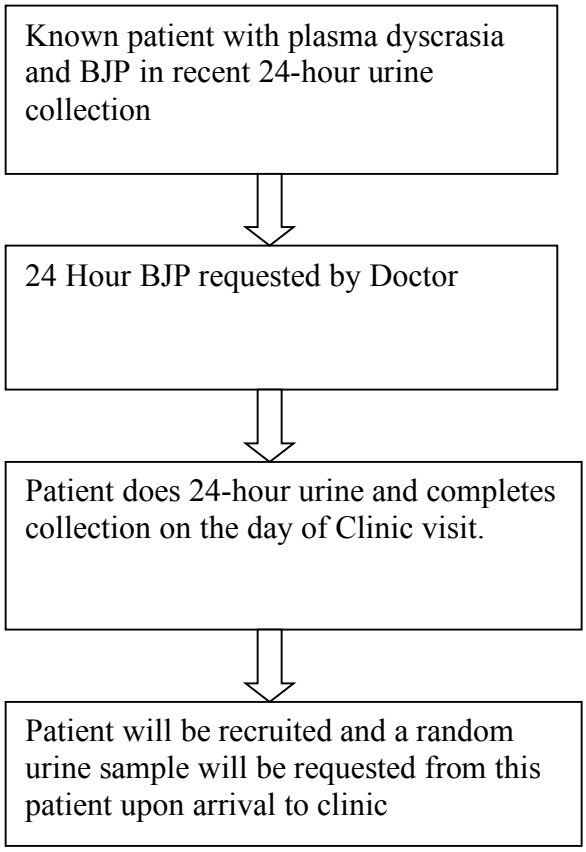
Sample size

This study will be regarded as a pilot study and the sample number is approximately 100 patient samples. A statistician was consulted with regards to sample size determination

Inclusion/Exclusion criteria

Included are the adult patients that attend the KEH Haematology clinic that have a plasma dyscrasia and a positive BJP urine on most recent UPEP.
Excluded are patients less than 18 years.

Sampling strategy
Informed consent will be obtained from all study participants (see appendix 1 Consent form- English and isiZulu version will be made available). Ethical clearance submitted to Biomedical Research Ethics Committee



Flow diagram showing sampling strategy for random urine collection

Data collection strategy and methods
Participants will collect random urine samples as per instructions provided (see appendix 2). Both samples will be submitted to the laboratory, however only the 24-hour collection as requested by the physician will be analysed and resulted by the routine laboratory for clinical patient care. Aliquots of both the random specimen and 24-hour urine sample will be analysed for total protein and creatinine (to ensure complete collection). The random sample will then be

frozen and stored at -70 C for a maximum of one month (stability as per manufacturer- Sebia) until UPEP is performed. Participants mass and height will also be recorded.
For both random and 24 hr urine collections: Urine protein electrophoresis will be performed using the Sebia BJP kits run on the Sebia Hydrasys. Quantitation of the M spike on UPEP (BJP) will be performed by densitometric scan of the UPEP gel. Total urine protein quantitation will be performed on the Siemens Advia 1800 chemistry analsyer using a dye binding Pyrogallol red, which complexes with proteins in anacid environment containing molybdate ions. The resulting blue-colored complex is read via spectrophotometric method. Urine creatinine will be analysed on the Siemens Advia 1800 chemistry analsyer using the modified kinetic Jaffe method. BJP: creatinine ratio will be calculated and the 24-hour BJP estimation will then be calculated using each of the following equations:
Twenty-four–hour BJP excretion calculated in 3 different ways:
1. Submitted BJP (mg) = %BJP X UTP (mg/dL) X 24-hour urine volume (dL)
2. Normalized BJP (mg) = %BJP X UTP (mg/dL)/UCR (mg/ dL) X patient's mean 24-hour creatinine excretion (mg)
3. Estimated BJP (mg) = % BJP X UTP (mg/dL)/UCR (mg/dL) X patient's weight-based expected 24-hour creatinine excretion (mg)[5]
Data will be collected from the patients' files and will be captured into an electronic database example Windows Excel and subsequently transferred to a statistical program for analysis. These programs will be password protected.
Clinical variables extracted from the charts include: hospital number, date of birth, height (cm), weight (kg), diagnosis, gender, race and age.

Statistical analyses
BJP quantitation values for 24-hour verse estimations from the random urine collections will then be compared using regression analysis and other relevant statistical analyses (Students t test)
The BJP results for each equation for the random urine specimens will be compared to the 24-hour urine BJP results also using regression analysis and Bland Altman plots.

Study period
The study period will be approximately 1 year January 2016-December 2016.

Limitations
Random specimens in comparison to timed or early morning urine collections are more prone to dilution of the specimen when collection occurs soon after the patient has consumed fluids. However, using a random sample is more convenient for the patient. Literature available on this topic is restricted.

Ethical considerations

This study has minimal ethical issues as it involves a non-invasive procedure of urine collection. This study will have no direct immediate impact on patients and will not affect their treatment. Patient confidentiality will be maintained at all times. Data will be collected on a password protected computer and the primary investigator will be the only person with access to it. Patients will be identified by hospital numbers and their identities will not be revealed.

Supervision and collaboration:

This research project will be performed under the supervision of Dr Verena Gounden, consultant chemical pathologist at the department of chemical pathology, and in collaboration with Dr Nadine Rapiti, Head of Department of Haematology at King Edward.

Appendix 2: The Guidelines for Authorship for the Journal selected for submission of the manuscript

CLINICAL BIOCHEMISTRY

Official Journal of the Canadian Society of Clinical Chemists

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Glossary

Please supply, as a separate list, the definitions of field-specific terms used in your article.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

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Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower- case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Maximum length of submissions

Full length articles should not exceed 4000 words (maximum 40 references), and up to 6 tables and/or figures with short communications comprising up to 1500 words of text, maximum 15 references, and two illustrative items (Tables and/or Figures). Letters will be classified as Case Reports (provide novel insight into disease mechanisms or diagnostic applications). Laboratory Notes (technical evaluation or important insight into analytical methodology), or Letters to the Editor (focused on a specific article that has appeared in Clinical Biochemistry within 4 weeks of print issue date of article). For all 3 types of letters listed above, the text should not exceed 500 words, with no abstract, a maximum of 1 table or figure and up to 5 references. Review Articles and Special Reports may exceed the word and reference limit for Full length articles as per the comprehensive nature of these articles. However, both of these articles (Reviews and Special Reports) will still require an abstract (unstructured, 250 word maximum). Editorials and Opinion pieces will not require an abstract and will be limited to 2000 words and up to 20 references.

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Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

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Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
[2] Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304. Reference to a website:
[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).

Reference to a dataset:
[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/ xwj98nb39r.1.

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Appendix 3: Ethical approvals

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 April 2016

Dr A Reddy (214585580)
Discipline of Chemical Pathology
School of Laboratory Medicine and Medical Sciences
Ashandree.reddy@nhls.ac.za

Protocol: Comparison of 24 hour urine **verse a** random **urine** sample for determination and quantification of Bence Jones protein.
Degree: MMed
BREC reference number: BE509/15

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 14 December 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 01 April 2016 to queries raised on 16 March 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval.**

This approval is valid for one year from **05 April 2016.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **10 May 2016.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: gounden1@ukzn.ac.za
cc postgrad: dudhrajhp@ukzn.ac.za

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Email: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management (HKRM)

Reference: **HRKM88/16**
KZ_2016RP19_462

01 April 2016

Dear Dr A Reddy
(University of KwaZulu-Natal/ National Health Laboratory Service)

Subject: Approval of a Research Proposal

1. The research proposal titled **'Comparison of 24 hour urine verse a random urine sample for determination and quantification of Bence Jones protein'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 01/04/16.

Fighting Disease, Fighting Poverty, Giving Hope

Appendix 4: Data collection tools and consent forms

Procedure to collect random urine sample

The 24-hour urine collections are usually completed on the day the patient comes to haematology clinic. On arrival, a sterile jar will be provided to patients to collect the sample of urine following the last void for the 24-hour urine collection.

Instruction on how to collect the urine:
Step 1
Wash your hands with soap and water.
Step 2
Open the small sterile specimen jar
Avoid contamination by not touching the inside of the jar or the jar lid.
Step 3
Void urine into the container; ensure that the container is at least half filled with urine.
Step 4
Give both the 24-hour and random urine samples to the nurse.

isiZulu transltion

Isithasiselo 2

Ukuthathwa komchamo emahoreni angama-24 kuvame ukuba kuphothulwe ngosuku isiguli esifika ngalo emtholampilo we-haematology. Uma zifika iziguli, zizonikwa ujeke ongenamagciwane ukuba zifake umchamo emva kokuthathwa komchamo wokugcina wamahora angama-24.

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Hlamba izandla ngamanzi nensipho.
Okwesibili
Vula ujeke omncane wokuthatha umchamo
Ungalithinti ingaphakathi noma isivalo sikajeke.
Okwesithathu
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Practice Number: 5200296

Informed Consent form for early morning spot urine collection for Bence Jones protein

This Informed Consent Form is for men and women who attend the haematology clinic at King Edward V11 Hospital, who we are inviting to participate in research on plasma dyscrasias. The title of our research project is "Spot the Bence Jones"

I am Dr Ashandree Reddy, working for the National Health Laboratory Services. We are doing research on plasma dyscrasias. I am going to give you information and invite you to be part of this research.
There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.
Plasma dyscrasias include diseases like multiple myeloma and amyloidosis which are serious and life-long diseases. A 24-hour urine collection for a protein called Bence Jones is done for patients with these types of disease to help the doctor to monitor a patient on treatment. Collecting a 24-hour urine sample, example from 6am to 6am the next morning is difficult. We are doing this research to see if we can use just one early morning urine sample in place of a 24-hour urine collection.

This research will involve an early morning spot (one sample) urine sample on the day that you are doing your 24-hour urine collection.

Your participation in this research is entirely voluntary. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

We will collect your early morning urine specimen each time you do your routine 24-hour urine collection for Bence Jones protein over a period of approximately 3 months. At the end of the research, in approximately 1 year, any leftover urine samples will be destroyed. The samples will only be used for this test.
The samples will be collected on the day of your usual clinic visit so there is no extra visits or time needed for this project.

Your participation is likely to help us find the answer to the research question and may benefit future generations in that they may have to simply collect a single early morning urine sample rather than a 24-hour collection.

The information that we collect from this research project will be kept confidential. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information.

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____
Day/month/year

If illiterate
A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ **AND** **Thumb print of participant**

Signature of witness _____

Date _____
Day/month/year

Statement by the researcher/person taking consent
I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:
1. An additional early morning spot urine collection to be done on the same day as the 24-hour urine collection.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.
Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____
Day/month/year

ACADEMIC COMPLEX BUSINESS UNIT

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Isithasiselo 1

Ifomu lemvume yokuthatha umchamo ukuze kuhlolwe iphrotheyini i-*Bence Jones*

Leli fomu lemvume elabesilisa nabesifazane abaya emtholampilo we-haematology esiBhedlela i-King Edward VIII abamenywa ukuba babe yingxenye yocwaningo lwe-*plasma cell dyscrasias*. Sanibona, igama lami nginguDkt. Ashandree Reddy, osebenza e-National Health Laboratory Services. Senza ucwaningo lwe-*plasma cell dyscrasias* (uhlobo lomdlavuza wegazi). Ngizoninika ulwazi bese nginimema ukuba nibe yingxenye yocwaningo.

Kungahle kube namagama eningawaqondi. Ngiyocela ningimise uma kukhona lapho nisala khona, ngiyobe sengicacisa lapho. Uma uba neminye imibuzo kamuva, ukhululekile ukungibuza.

I-*Plasma dyscrasias* ibandakanya izifo ezinjenge-*multiple myeloma* kanye ne-*amyloidosis* okuyizifo ezibucayi futhi ezihlalayo. Ukuthathwa komchamo isikhathi esingamahora angama-24 kutholwa iphrotheyini ebizwa nge-*Bence Jones* kwenziwa ezigulini ezinalezi zifo ukusiza udokotela ukuba akwazi ukubheka iziguli ezelashwayo. Ukuthathwa kwesampula lomchamo wamahora angama-24 kunzima, isibonelo nje, kusukela ngehora lesi-6 ekuseni kuye ehoreni lesi-6 ekuseni ngosuku olulandelayo. Senza lolu cwaningo ukuze sithole ukuthi singakwazi yini ukuba sithathe isampula elilodwa lomchamo esikhundleni sokuthatha umchamo wamahora angama-24.

Lolu cwaningo luzobandakanya ukuthathwa komchamo ngelanga osuke uzothathwa ngalo umchamo wamahora angama-24.

Siyothatha umchamo wakho zikhathi zonke uma uzothathwa umchamo wamahora angama-24 wephrotheyini i-*Bence Jones* esikhathini esiyizinyanga ezi-3. Ekupheleni kocwaningo, okuyoba emva konyaka, ayolahlwa onke amasampula omchamo ayobe esele. La masampula ayosetshenziselwa lolu cwaningo kuphela. Amasampula ayothathwa ngosuku osuke uze ngalo emtholampilo ukuze ungabi nezinsuku ezengeziwe ozozizela le phrojekthi.

Ukuzibandakanya kwakho kungase kusisize ukuba sithole izimpendulo zombuzo wocwaningo futhi kungasiza izizukulwane ezizayo ngokuthi seziyothathwa isampula lomchamo elilodwa kunokuthi uthathwe isikhathi esingamahora angama-24.

Ulwazi esiluqoqa kulolu cwaningo luyohlala luyimfihlo. Yonke imininingwane yakho iyoba nenombolo esikhundleni segama lakho. Abacwaningi kuphela abayokwazi inombolo yakho futhi siyoluvalela lolo lwazi.

Uyazikhethela ukubamba iqhaza kulolu cwaningo. Noma ungakhetha ukuzibandakanya nocwaningo noma ukhethe ukungazibandakanyi nocwaningo, akukho lutho oluzoshintsha noma ozokwephucwa khona ngokuza kulo mtholampilo. Ungabuye ushintshe umqondo wakho uyeke ukuba yingxenye yocwaningo. Kuyilungelo lakho okumele lihlonishwe.

Uma ufisa ungangibuza noma yimuphi umbuzo omayelana nocwaningo. Unayo imibuzo?

Isitifiketi Sencwadi Yemvume

Ngilufunde lonke ulwazi olungenhla, noma ngifundelwe lona. Ngibe nethuba lokubuza imibuzo ngalo, yonke imibuzo engiyibuzile ngenelisekile ngokuphendulwa kwayo. Ngiyavuma ukubamba iqhaza kulolu cwaningo.

Igama Lombambiqhaza _____

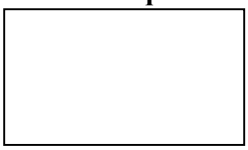
Isiginesha Yombambiqhaza _____

Usuku _____ Usuku/inyanga/unyaka

Kongakwazi ukufunda nokubhala

Bengikhona kufundwa ngobunyoninco incwadi yesicelo semvume kongaba ngumbambiqhaza, naye unikeziwe ithuba lokubuza imibuzo. Ngiyaqinisekisa ukuthi uzikhethele ngokwakhe ukubamba iqhaza ocwaningweni.

Igama likafakazi_____ KANYE nokucindezela isithupha kombambiqhaza



Isiginesha kafakazi_____

Usuku _____ Usuku/inyanga/unyaka

Isitatimende somcwaningi/umuntu ocela imvume

Ngimfundele lonke ulwazi lowo ongaba ngumbambiqhaza, ngawo onke amandla ami ngiqinisekisile ukuthi umbambiqhaza ukuqonda konke ukuthi kuzokwenziwa lokhu okulandelayo:

1. Konke ukuthathwa komchamo okwengeziwe kuyokwenziwa ngosuku olufanayo lokuthathwa komchamo emahoreni angama-24.

Ngiyaqinisekisa ukuthi umbambiqhaza wanikwa ithuba lokubuza imibuzo mayelana nocwaningo, nayo yonke imibuzo ayibuza yaphendulwa ngokufanelekile. Ngiyaqinisekisa ukuthi umbambiqhaza akaphoqwanga ukunikeza imvume kepha uzivumele yena ngokwakhe. Ikhophi yale ncwadi yemvume unikeziwe naye umbambiqhaza.

Bhala igama lomcwaningi/umuntu ocela imvume_____

Isiginesha yomcwaningi /umuntu ocela imvume_____

Usuku _____

Usuku/inyanga/unyaka

Lolu cwaningo lubukiwe lwase luvunywa yi-UKZN Biomedical research Ethics Committee (inombolo yokuvunywa_____).Uma kuba khona izinkinga noma imibuzo ungaxhumana nomcwaningi ku-0312402558 noma i-UKZN Biomedical Research Ethics Committee, imininingwane yokuxhumana imi kanje: BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
University of KwaZulu-Natal
Private Bag X 54001, Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2602486 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Appendix 5: Raw data

	Summary Data Sheet												
	Study No	Diagnosis	Age (years)	Gender	Race	BMI kg/m2	BJP mg/24hr	IMWG response	% BJP	JP: creat ratio (mg/mmol)	E 1 mg/24hr	E 2 mg/24hr	IMWG response
1	MM001	MM	58	F	B	22,5	2520	2	67,8	125,6	1256,3	949,5	2
2	MM002	MM	74	M	B	23,1	1080	2	50	128,6	1286,2	1705,5	2
3	MM004	MM	57	F	B	24,6	3430	2	82,3	163,6	1636,4	1562,3	2
4	MM010	MM	61	M	B	15,4	690	2	53	90,0	899,6	795,3	2
5	MM011	MM	59	F	B	36,7	LDL	0	40,6	35,1	350,8	437,3	2
6	MM014	MM	54	F	B	24,9	6800	2	67,9	471,3	4712,5	4499,2	2
7	MM020	MM	59	M	B	23,5	LDL	0	15,5	46,1	460,5	447,8	2
8	MM021	MM	58	M	B	33,1	380	2	26,2	59,8	598,0	1046,6	2
9	MM022	MM	63	M	B	28,7	LDL	0	25,2	39,7	397,1	575,8	2
10	MM024	MM	47	M	B	26,3	14400	2	52,2	714,3	7142,9	9597,7	2
11	MM025	MM	56	F	B	29,8	8580	2	86	513,7	5137,1	5790,0	2
12	MM028	MM	56	F	B	32,8	9240	2	81,6	528,6	5286,4	5888,2	2
13	MM030	MM	53	F	B	33,3	380	2	53	39,4	393,5	412,3	2
14	MM031	MM	65	M	B	22,3	2660	2	57,1	191,8	1917,8	2034,4	2
15	MM059	MM	57	F	I	35,4	250	2	35	0,0	0,0	0,0	0
16	MM060	MM	47	F	B	20,3	2480	2	81,9	448,2	4481,6	3743,9	2
17	MM061	MM	49	M	B	30,4	680	2	37	21,2	211,8	269,7	2
18	MM062	MM	49	F	B	27,4	1200	2	55	162,8	1628,2	1403,4	2
19	MM063	MM	55	M	B	35,5	3600	2	69	95,7	956,7	1945,2	2
20	MM064	MM	48	M	B	26,2	290	2	34,5	29,7	297,0	383,4	2
21	MM065	MM	47	F	B	26,6	1610	2	75,9	54,9	549,5	728,6	2
22	MM066	MM	56	F	B	27,0	6150	2	77	708,8	7087,6	6484,8	2
		Summary Data Sheet											
		NOTES:	MM: Multiple myeloma										
			M:Male F: Female										
			B: Black I: Indian										
			E1: Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) X10										
			E2: Estimated 24-hour BJP (mg/24hour) = Urine BJP/Creatinine ratio (mg/mmol) x 15mg/kg for women or x 20mg/kg for men (convert mg/kg to mmol/kg by multiplying 0.00884)										
			LDL: Lower than Detectable limit										
			Red: Random urine										