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ABSTRACT

Background: Multiple Myeloma (MM) is a haematological malignancy with increasing global incidence. Diagnosis of MM should be initiated at the primary care level to achieve the best patient outcome. However, this can be delayed due to nonspecific presenting symptoms, such as back pain and fatigue.
Objectives: The aim of this study was to investigate if commonly requested blood tests could indicate MM in primary care and potentially lead to earlier diagnosis.
Design and Methods: This retrospective observational study involved an audit of clinical and laboratory data from 109 MM patients, including patients with Active MM (N = 53), Smouldering MM (N = 33), and Free light chain MM (N = 23).
Results: Of the 16 potential biomarkers investigated, the most promising indicator for early detection of active MM and Smouldering MM was an increased Calculated Globulin (CG). The median CG for patients with active MM (50 g/L) was 78.6% higher than the healthy control group (28 g/L). Smouldering MM patients had a median CG value (38 g/L), which was 35.7% higher than the control group. Of interest, the median CG result was only 16.7% higher in the control group than in the free light chain MM group, suggesting CG would not be as effective at detecting this subtype.
Conclusions: CG is derived from Total Protein and Albumin data, which are commonly measured in routine liver function profiles, thus there is no additional test or cost requirement. Based on these data, CG has potential as a clinical biomarker to support early detection of MM at the primary care level and allow for appropriate targeted investigations.

1. Introduction

1.1. Prevalence and subcategories of Multiple Myeloma

Multiple Myeloma (MM) is the second most common haematological cancer, accounting for approximately 1% of all cancers and approximately 10% of all haematological malignancies [1]. The incidence of MM continues to increase, with a recent study reporting that between 1990 and 2016, global incidence rose by 126%, and MM-associated deaths rose by 94% [2].

Within MM, there are different subcategories with varying clinical significance. Monoclonal Gammopathy of Undetermined Significance (MGUS) is a premalignant condition from which MM and other lymphoproliferative disorders can evolve [1,3]. Smouldering Multiple

Abbreviations: AUC, Area Under Curve; CG, Calculated Globulin; CREC, Cork Research Ethics Committee; CRP, C-Reactive Protein; CUH, Cork University Hospital; ESR, Erythrocyte Sedimentation Rate; FLC, Free Light Chain; FLCMM, Free Light Chain Multiple Myeloma; GDPR, General Data Protection Regulation; GP, General Practitioner; Hb, Haemoglobin; HCT, Haematocrit; IFE, Immunofixation Electrophoresis; IMWG, International Myeloma Working Group; LDH, Lactate Dehydrogenase; LIS, Laboratory Information System; MRN, Medical Record Number; MCV, Mean Corpuscular Volume; MGUS, Monoclonal Gammopathy of Undetermined Significance; MM, Multiple Myeloma; ROC, Receiving Operating Characteristic; SPEP, Serum Protein Electrophoresis; SMM, Smouldering Multiple Myeloma; TP, Total Protein; WHO, World Health Organization.

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Table 1
Patient group demographics and characteristics.

Group	Male Patients	Female Patients	Mean Age (years)	Age Range (years)	Median Paraprotein Size at Diagnosis (g/L)	Paraprotein Size Range (g/L)
MM	34	19	70.8	37–90	23.7	3.4–76.4
SMM	22	11	67.5	36–90	17.9	6.1–42
FLCMM	11	12	62.7	37–84	2.4	0.2 - 16.8
Control	34	19	68.6	35–88	N/A	N/A

Data is presented for the three test groups, including active Multiple Myeloma (MM), smouldering Multiple Myeloma (SMM), free light chain Multiple Myeloma (FLCMM) and the control group.

Myeloma (SMM) refers to the transitional phase between MGUS and active MM. Patients with SMM are generally asymptomatic but have a high risk of progression to symptomatic/active MM, with approximately 10% of patients progressing per annum in the first five years after diagnosis [3]. SMM patients identified as high-risk will develop clinical symptoms and end-organ damage within two years of diagnosis. Active MM can affect multiple tissues, organs and systems, including bones, kidneys, blood, and immune systems [4]. MM is characterized by the neoplastic proliferation of a single clone of plasma cells, often resulting in the overproduction of monoclonal protein (M−protein) also known as paraprotein [4]. The majority of MM patients produce both paraproteins as well as monoclonal free light chains [5]. However, some MM only produce monoclonal free light chains with no detectable paraprotein. These are known as Free Light Chain Multiple Myeloma (FLCMM).

Early clinical detection and intervention can significantly improve patient well-being and prognosis, thus, identifying clinical biomarkers that could indicate SMM, as well as MM and FLCMM at an earlier stage, would be of great clinical utility and would support better patient outcomes [4].

1.2. Assessment of monoclonal proteins in peripheral blood

Diagnosis of MM can be aided by detecting M−protein with methods such as serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE) and free light chain (FLC) assays [6]. As General Practitioners (GPs) have a low index of suspicion for MM, they are unlikely to request these disease-specific tests [7]. Of interest in a 2016 Irish study GPs indicated a lack of clear guidance on when it is appropriate to request specific M−protein tests [8]. A suitable biomarker within the routine panel of laboratory investigative tests could potentially help guide GPs on when MM-specific tests should be requested.

1.3. Importance of diagnosis at the primary care level

To achieve the best outcome for a patient, early diagnosis of MM at primary healthcare stage is desirable. Howell et al (2017) reported that MM patients diagnosed via the emergency route were more likely to have clinically advanced disease, require additional radiotherapy or surgery, and have the poorest survival rates [8]. Studies have shown that MM patients with a delay of 6 months or greater have increased complications such as anaemia, bone disease, renal impairment and reduced disease-free survival [7]. A 2016 UK based study investigating referral times of MM patients found that of patients who initially presented to a GP, 33% waited for over a year for a specialist referral [7]. Of interest, in an earlier study it was reported that the proportion of patients that had multiple GP visits prior to referral was highest in the MM cohort than the other 23 cancers investigated, with 50.6% having three or more pre-referral consultations [9].

Delays in diagnosis may be due to nonspecific symptoms such as anaemia and bone pain. A 2009 study involving the data of 5483 MM patients found substantial variation in their diagnostic intervals, with a range of 1 to 365 days and a mean of 137 days [10]. An earlier indication of MM at primary care could potentially result in a significant reduction of the total interval from symptom onset to diagnosis for these patients [11].

The primary objective of this study was to identify a biomarker(s) from routinely requested biochemistry and haematology blood panels that could potentially be utilized as early indicators of MM, SMM, and/or FLCMM at the primary care level. All analytes included in the study, with the exception of Calculated Globulin (CG) are currently reported in the biochemistry or haematology laboratories of the study site, Cork University Hospital (CUH), Ireland, and are routinely requested by GPs. The globulin values were calculated from two of these targets, Total Protein (TP) and albumin.

2. Methods

2.1. Ethical approval and selection of sample groups

This study was based on a retrospective observational audit of clinical and laboratory data from patients diagnosed with MM, SMM, and FLCMM. These data were retrieved from the biobanks of CUH, in accordance with GDPR guidelines. The study was conducted, and all data was collected, following approval from Cork Research Ethics Committee (CREC), reference number: ECM 05/2021 PUB.

The three patient groups (MM, SMM, and FLCMM) were identified from those who attended haematology clinics in CUH in 2020. Hospital records were accessed to extract patient information relating to the MM subcategory, date of diagnosis, and details of electrophoretic profiles. A control group included a cohort of patients with similar demographics to the MM group, with no history of MM diagnosis and a negative paraprotein screen (Table 1).

2.2. Assessment of biological variables

The analytes evaluated in this study included: TP, albumin, Calculated Globulin (CG), calcium, creatinine, lactate dehydrogenase (LDH), haemoglobin (Hb), ferritin, C- reactive protein (CRP), mean corpuscular volume (MCV), neutrophils, platelets, eosinophils, erythrocyte sedimentation rate (ESR), red blood cell count (RBC), and haematocrit (HCT). The choice of analytes to include was primarily based on the International Myeloma Working Group (IMWG) guidelines [1]. As CG is not measured in the test site (CUH), it was determined by subtracting the albumin from the TP concentration. The serum TP (biuret method) and Albumin (bromocresol green method) were measured by Beckman AU5832 biochemical analyser.

For patients in the three test groups, laboratory data prior to treatment was accessed and recorded (up to 30 days prior to diagnosis) to rule out any impact from treatment. For patients in the control group, excluding any MGUS or undiagnosed MM patients was important, thus data collected represented patients with no history of MM on the same date or up to 30 days prior to a negative SPEP screen.

2.3. Calculation of CG reference interval

As CG is not reported at the study site to date, there was no population-specific reference interval available. To create a reference interval, the Laboratory Information System (LIS) was accessed using

Table 2
Median values of analytes for MM, SMM, FLCMM, and Control groups.

Test		Reference Range	MM (N = 53)	SMM (N = 33)	FLC (N = 23)	Control (N = 53
Total protein (g/L)		62 to 82	86	77	67	71
Albumin (g/L)		35 to 52	37	39	43	43
Globulin (g/L)		N/A	50	38	24	28
Calcium (mmol/L)		2.1 to 2.65	2.42	2.36 (n = 28)	2.43	2.37 (n = 51)
Creatinine (μmol /L)	Male	64 to 104	97.5 (n = 34)	85.0 (n = 21)	104.0 (n = 11)	84.5 (n = 34)
	Female	49 to 90	74.0 (n = 19)	67.0 (n = 12)	69.5 (n = 12)	67 (n = 19)
LDH (units/L)		220 to 450	359.0 (n = 51)	343.5 (n = 27)	426.0 (n = 20)	452.0 (n = 8)
CRP (mg/L)		0 to 5	4.7 (n = 46)	7.3 (n = 9)	7.6 (n = 19)	4.3 (n = 23)
Haemoglobin (g/dl)	Male	13.0 to 17.0	10.5 (n = 34)	13.5 (n = 21)	11.1 (n = 11)	14.2 (n = 34)
	Female	11.7 to 15.9	10.4 (n = 19)	12.1 (n = 12)	10.2 (n = 12)	13.1 (n = 19)
Ferritin (ug/L)	Male	17 to 320	446.0 (n = 23)	82.5 (n = 10)	335.0 (n = 4)	138.5 (n = 12)
	Female	11 to 307	242.0 (n = 10)	90.0 (n = 7)	327.5 (n = 8)	77.0 (n = 8)
MCV (f/L)		80 to 96	90.4	91.2	89.0	89.1
ESR (mm/hr)	Male	1 to 10	83.5 (n = 10)	24.0 (n = 5)	13.0 (n = 4)	2 0.0 (n = 19)
	Female	1 to 20	44.0 (n = 3)	40.0 (n = 2)	29.0 (n = 4)	9.5 (n = 8)
Neutrophils (10^9/L)		1.4 to 6.6	3.65	3.23	3.84	4.03
Platelets (10^9/L)		140 to 440	222	229	230	235
Eosinophils (10^9/L)		0.04 to 0.4	0.08	0.13	0.12	0.15
RBC (10^{12}/L)	Male	4.2 to 5.6	3.24 (n = 34)	4.27 (n = 21)	3.65 (n = 11)	4.65 (n = 34)
	Female	3.9 to 5.3	3.40 (n = 19)	3.89 (n = 12)	3.19 (n = 12)	4.20 (n = 19)
HCT (L/L)	Male	0.38 to 0.49	0.291 (n = 34)	0.387 (n = 21)	0.325 (n = 11)	0.421 (n = 34)
	Female	0.35 to 0.46	0.302 (n = 19)	0.349 (n = 12)	0.292 (n = 12)	0.390 (n = 19)

Reference ranges and median values for biological targets were measured across three test groups, active Multiple Myeloma (MM), smouldering Multiple Myeloma (SMM), free light chain Multiple Myeloma (FLCMM), and a control group. Abbreviated analytes are lactate dehydrogenase (LDH), C- reactive protein (CRP), mean corpuscular volume (MCV), erythrocyte sedimentation rate (ESR), red blood cell count (RBC), and haematocrit (HCT). Creatinine, Haemoglobin, Ferritin, RBC and HCT were subdivided into male and female as they have separate reference intervals. The frequency of test requests is also displayed, where N = the total number of patients in each group and n = the number of patients for whom the analyte data was available.

Cognos Impromptu Administrator IBM software (version 7.5), to compile a list of all patients with a normal SPEP pattern within a one-month study period (June 2020). Duplicate Medical Record Numbers (MRNs) were removed to ensure patients only appeared once. For the first 120 patients, data relating to TP and albumin were recorded and used to calculate the Globulin (g/L) for each patient. MedCalc (v20.113) was used to analyze the data, which was reported as the frequency of CG levels (g/L) and CG reference interval data. Reference intervals were available for all other analytes.

2.4. Statistical analysis

Statistical analysis was performed using the statistical package SPSS (28.0.1.1), and Microsoft Excel add-on software (Analyse-it (6.15)). For each analyte each test group was compared to the control group.

Reference intervals for each analyte can be seen in Table 2. As Hb, HCT, RBC, creatinine, ESR, and ferritin analytes have different reference ranges for males and females, these groups were subdivided for statistical analysis.

A non-parametric Mann-Whitney *U* test was performed to assess whether two groups were likely to derive from the same population. If the Mann-Whitney *U* results of any one of the analytes produced a *p*-value < 0.05, the null hypothesis that there is no difference between the routine lab results of patients with and without MM could be rejected.

The effect size estimate of each Mann-Whitney *U* test with a *p*-value < 0.05 was calculated using the z score generated in the Mann-Whitney *U* test and the sample size for that comparison. The calculation used was:

$$r = \frac{Z}{\sqrt{N}}$$

where r is the effect size estimate, Z is the z score generated by the Mann-Whitney *U* test, and N is the size of the study. *U* reflects the difference between the two rank totals.

All analytes associated with a *p*-value below the threshold of 0.05 were eligible for consideration as a statistically significant potential indicator of MM, SMM or FLCMM.

Receiving Operating Characteristic (ROC) curves were generated, to assess CG's diagnostic ability. In addition, decision threshold graphs were used to determine the optimal threshold to discriminate MM from healthy cases. The area under the curve (AUC) is a measure of the CG ability to distinguish between two groups. The closer the ROC curve is to the upper left corner of the graph, the higher the accuracy of the test, i.e an AUC of 1.0 would have sensitivity = 1 and the false positive rate = 0 [12].

3. Results

3.1. Group demographics and electrophoretic profiles

The test groups consisted of MM patients (N = 53), SMM patients (N = 33), and FLCMM patients (N = 23), while the control group had 53 patients for analysis. Group demographics and paraprotein profiles, as determined by SPEP, are given in Table 1.

3.2. Measurement of biological targets

Median values for each target analyte, as determined for each patient group and the control group are presented in Table 2. Data for some analytes were available for all patients, while other analytes were requested less frequently. For example, data for TP, albumin, creatinine, Hb, MCV, neutrophils, platelets, eosinophils, RBC, and HCT were available on every patient in all groups. ESR was available in the lowest frequency, and the resulting sample size was deemed too small for analysis.

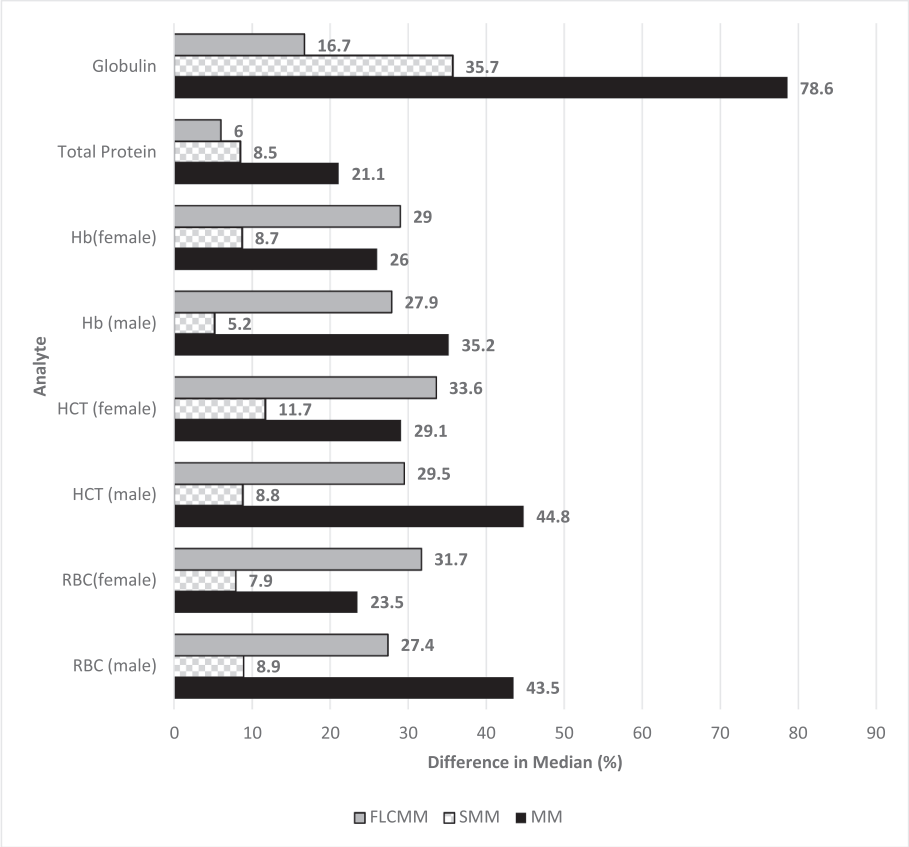

Fig. 1. Percentage difference of the medians of analytes from the three test groups relative to the medians of the control group analytes.

3.3. Comparison of mean ranks – Mann-Whitney U test

Based on mean rank values, five of the analytes (CG, TP, Hb, HCT, and RBC) were significantly different ($p < 0.05$) to the control group across all patient groups, including MM, SMM, and FLCMM (Fig. 1).

Fig. 2 depicts the effect size estimates of each of these comparisons, which signifies the magnitude of the difference between groups [13].

The five analytes which gave a statistically significant result ($p < 0.05$) in the Mann-Whitney *U* test are depicted in this graph, in which the percentage difference in the medians of the analytes from the three test groups relative to the control group is displayed. The three test groups are active Multiple Myeloma (MM), smouldering Multiple Myeloma (SMM), and free light chain Multiple Myeloma (FLCMM). Haemoglobin (Hb), red blood cell count (RBC), and haematocrit (HCT) were subdivided into male and female as they have separate reference intervals.

The effect size of the five analytes, which were statistically significant ($p < 0.05$) in the Mann-Whitney *U* test are depicted. Haemoglobin (Hb), red blood cell count (RBC), and haematocrit (HCT) were subdivided into male and female as they had separate reference intervals.

As seen in Fig. 1 there is a 78.6% difference in the median CG values between the MM group (median = 50 g/L) and the control group (median = 28 g/L), U = 139, z = -8.006, $p = 0.0$, r = 0.78. There is a 35.7% difference in the median CG values between the SMM group (median = 38) and the control group (median = 28), U = 156.5, z = -6.387, $p = 0.0$, r = 0.69. There is a 16.7% difference in the median CG values between the FCMM group (median = 24) and the control group (median = 28), U = 332.5, z = -3.144, $p = 0.001$, r = 0.36.

3.4. CG reference interval and the optimal threshold value

For this study, 120 patients (n = 78 female, n = 42 male) covering a wide age range (18–82 years) were selected to create a CG reference interval. The frequency histogram of the CG reference values showed a normal distribution. Data analysis of the 120 CG values generated a 95% reference interval of 20.20–33.47 g/L.

To assess the diagnostic ability of CG to predict active MM, a ROC curve based on CG values from all of the MM patients (N = 53, positive cohort) and the control group (N = 53, negative cohort) was generated (Fig. 3A), and the area under the curve (AUC) determined to be = 0.95 ($p < 0.0001$).

A second ROC curve (Fig. 3B) was generated using the CG values from the SMM patients (N = 33, positive cohort) and the control group (N = 53, negative cohort). The AUC was determined as 0.911 ($p < 0.0001$).

In this curve, specificity (x-axis) is plotted against sensitivity (y-axis) to illustrate the ability of Calculated Globulin to discriminate between a healthy population, i.e., the control group, and the active Multiple Myeloma group (A) and Smouldering Myeloma Group (B).

The optimal threshold value to distinguish MM patients from control group subjects was 39 g/L. At this threshold sensitivity = 0.755, specificity = 1.000, positive predictive value (PPV) = 1.00, and negative predictive value (NPV) = 0.80. The optimal threshold value to distinguish between SMM and control patients was 33 g/L. At this threshold sensitivity = 0.788, specificity = 0.906, PPV = 0.84, and NPV = 0.87.

3.5. Potential of CG to aid earlier MM diagnosis

Of the 53 patients in the active MM group, only 23% had an initial diagnosis of MGUS and were monitored appropriately. The remaining 77% had already progressed to active MM at the time of diagnosis. Of interest, 5% of patients had no prior GP visit and therefore could not have been detected earlier at the primary level. 24% had prior GP visits but no TP as it was not part of the routine liver panel at the time, so

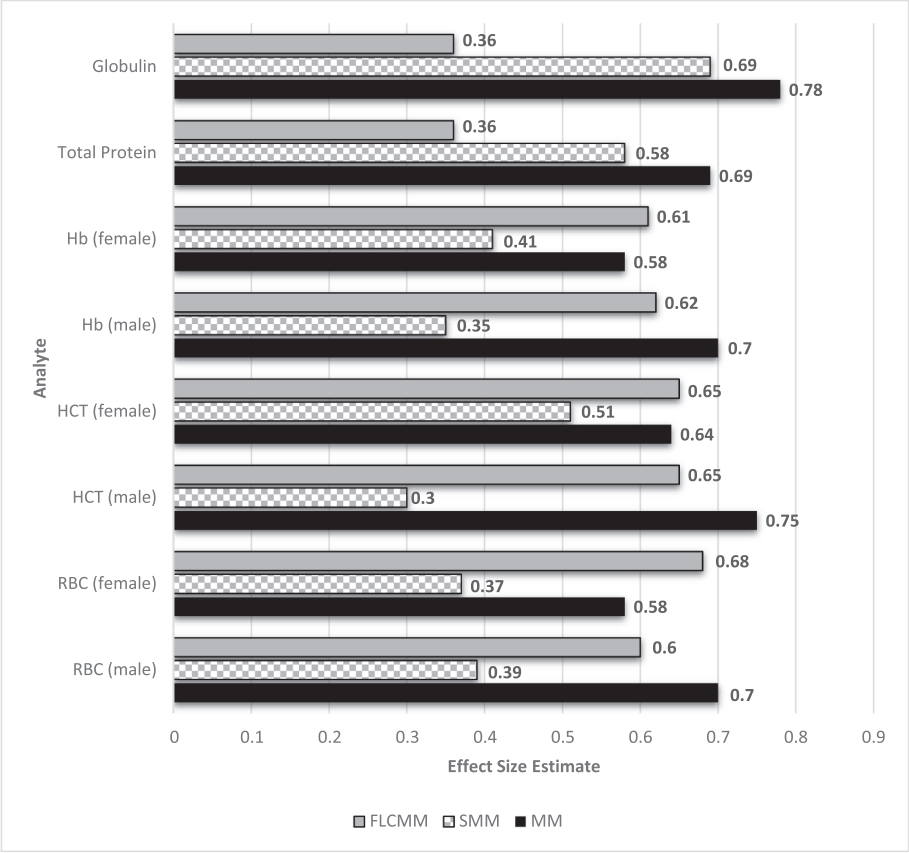

Fig. 2. The effect size of Mann-Whitney U tests comparing analytes from the three test groups to the control group.

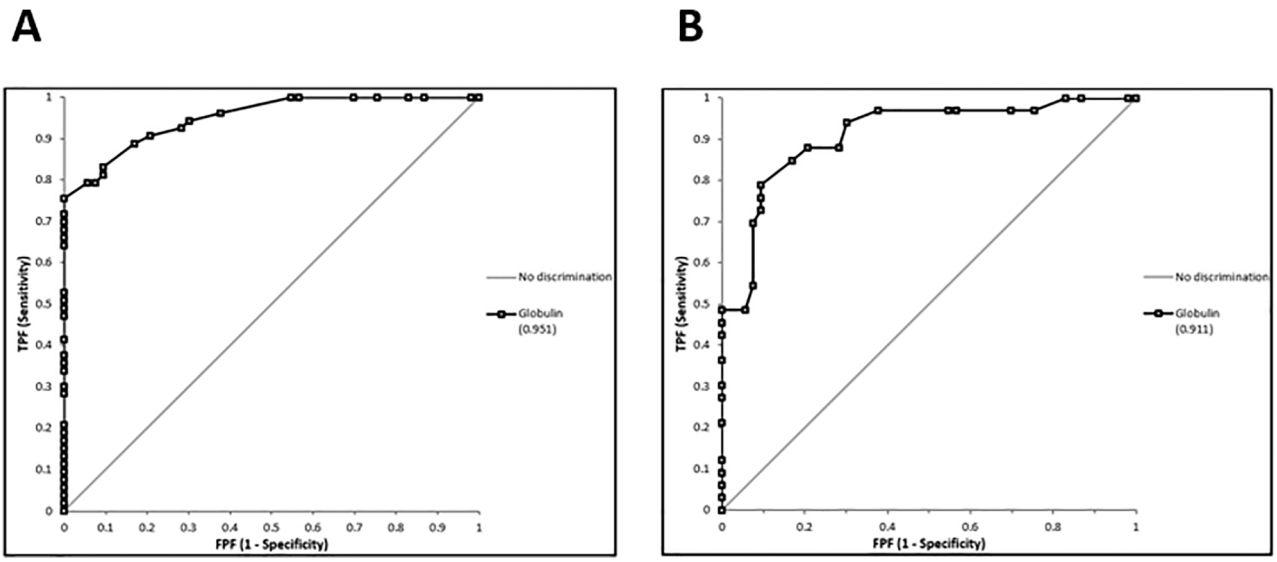

Fig. 3. Receiver Operating Characteristic (ROC) curve illustrating the diagnostic ability of Calculated Globulin to predict active Multiple Myeloma (A) and Smouldering Multiple Myeloma (B).

Globulin values could not be determined.

Of the remaining 29 patients in the active MM group, 79% had prior GP visits with a CG above the normal range (20.20–33.47 g/L), and 58% had a CG above the threshold of 39 g/L.

In these patients (41% of those not initially diagnosed as MGUS), their CG values were above the 39 g/L threshold in a range of 1 to 68 months prior to MM diagnosis, with a mean of 15 months.

4. Discussion

If left untreated, MM patients can develop renal dysfunction and skeletal complications, decreasing their quality of life and increasing mortality. For MM patients presenting at primary care with a low index of suspicion, a routinely requested biological target could support early disease detection [11].

Data from this study indicated a statistically significant difference (*p*

< 0.05) between the test group and the control groups for five biological targets, including TP, CG, Hb, RBC, and HCT, suggesting that these have potential as clinical biomarkers for indicating MM. Of these, CG had the most significant percentage difference in medians when comparing the control group to the MM (78.6% difference) and SMM (35.7% difference) groups. This suggests that, CG had the most potential to discriminate between healthy and MM populations. Noteworthy, CG meets the World Health Organization (WHO) requirements of a screening test [14,15].

Importantly, previous studies have demonstrated a strong correlation between CG and total immunoglobulin (Ig) concentration, making it a valuable tool for detecting abnormal Ig concentrations [16–20]. This correlation suggests that reporting CG may have a clinical utility in screening for paraproteinemia often associated with MM and SMM, as well as hypogammaglobulinemia associated with FLCMM [21].

Using the CG reference interval developed in this study, 83% of patients in the MM group and 78% of the patients in the SMM group had CG results greater than the upper end of the reference range. Some MM patients had a normal CG, which could be due to MM with relatively small paraproteins, non-secretory or oligosecretory MM, or MM in which significant immunosuppression in the globulin region counterbalances the size of the paraprotein [22]. It is also worth noting that paraproteins are not the only cause of elevated CG, as demonstrated by the 9% of patients in the control group who had CGs greater than the reference interval. This is likely due to diffusely raised immunoglobulin, which can be associated with infection [23].

In the case of the FLCMM group, there is no paraprotein present to cause an increased CG region. Instead, a decreased CG was expected due to the immunosuppression that often accompanies FLCMM. When comparing the FLCMM group to the control group, the percentage differences between the medians for Hb (females 29%, males 27.9%), RBC (females 31.7%, males 27.4%), and HCT (females 33.6%, males 29.5%) were considerably higher than the percentage median difference for CG (16.7%). This indicates that although we see the immunosuppression-associated reduction in CG for patients with FLCMM, the biomarkers associated with anaemia are a stronger early indicator of disease. Unfortunately, anaemia is a relatively nonspecific symptom and is unlikely to be particularly useful as an indicator of MM at the primary care level. Of the 23 patients in the FLCMM group, only 30% had a CG result lower than the reference interval. These results do not support CG as a valuable indicator of FLCMM unless significant immunosuppression is present. It is worth commenting on the fact that the sample size for FLCMM was relatively small in this study ($N = 23$), and thus a study involving a larger dataset may be warranted to determine if it would be of benefit to recommend SPEP on patients with low CG.

Based on a retrospective analysis, 77% of the active MM group were not diagnosed at the MGUS or SMM stage. It was also identified that 41% of these patients had CG above the 39 g/L threshold available at the primary care level an average of 15 months prior to diagnosis. This suggests that if CG data had been available, it may have led to earlier M−protein investigation and diagnosis.

It is also worth noting that while this study focused solely on detecting MM, CG measurement could also be relevant for earlier detection of other monoclonal gammopathies, such as Waldenstrom Macroglobulinaemia [24].

5. Conclusion

Although decreased CG was not determined to be a viable indicator of FLCMM, increased CG has potential as an early indicator of secretory MM and SMM. Implementing this inexpensive and readily available strategy could help reduce the diagnostic delay and allow appropriate targeted investigations for MM patients, as well as potentially indicate other paraproteinemias.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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