blood® Regular Article

LYMPHOID NEOPLASIA

Serum free light chains in a racially diverse population including African Americans and populations from South Africa

Luca Bertamini,[1,2,*] Jean-Baptiste Alberge,[1-3,*] David J. Lee,[1-4] Habib El-Khoury,[1,2] Sungjae Kim,[1,3] Grace Fleming,[1,2] Ciara Murphy,[1,2] Julia Colchie,[1,3] Maya I. Davis,[1,2] Jacqueline Perry,[1] Elizabeth D. Lightbody,[1-3] Sabine Allam,[1-3] Lindokuhle N. Goqwana,[5,6] Vinitha Philip,[6] Natalie Smyth,[7] Dhananjay Sakrikar,[8] Mark Perkins,[9] Stephen Harding,[9] Derek Troske,[8] Gad Getz,[3,10,11] Elizabeth W. Karlson,[2,12] Nikhil Munshi,[2] Kenneth C. Anderson,[2] Lorenzo Trippa,[13,14] Catherine R. Marinac,[1] Wenlong C. Chen,[7,15,16,†] Maureen Joffe,[15,†] and Irene M. Ghobrial[1,2,†]

[1]Center for Early Detection and Interception of Blood Cancers, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; [2]Department of Medicine, Harvard Medical School, Boston, MA; [3]Cancer Program, Broad Institute of MIT and Harvard, Cambridge, MA; [4]Department of Medicine, Massachusetts General Hospital, Boston, MA; [5]Division of Internal Medicine, Faculty of Health Sciences, School of Clinical Medicine, University of the Witwatersrand, Johannesburg, South Africa; [6]Clinical Haematology Unit, Department of Medicine, Faculty of Health Sciences, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa; [7]Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [8]Binding Site, Part of Thermo Fisher Scientific, Rochester, MN; [9]Binding Site Group, Part of Thermo Fisher Scientific, Birmingham, United Kingdom; [10]Center for Cancer Research and [11]Cancer Center and Department of Pathology, Massachusetts General Hospital, Boston, MA; [12]Department of Medicine, Brigham and Women's Hospital, Boston, MA; [13]Biostatistics, T.H. Chan School of Public Health, Harvard University, Cambridge, MA; [14]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Center, Boston, MA; [15]Strengthening Oncology Services Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; and [16]National Cancer Registry, National Health Laboratory Service, Johannesburg, South Africa

KEY POINTS

- Reference FLC values are applied globally but were established using serum from mostly White individuals.
- A κ/λ ratio between 0.686 and 2.10 applies to racially diverse populations and is consistent with known LC disease patterns.

Detection of light chain (LC) monoclonal gammopathies (MGs) traditionally relies on serum free LC (FLC) κ, λ, and their ratio (κ/λ) reference ranges based on a mostly White population. We investigated FLC values in a racially diverse population by screening 10 035 individuals for heavy chain MG, identifying 9028 negative cases whose FLC were measured. Participants included 4149 from the PROMISE study (United States, n = 2383; South Africa, n = 1766) and 4879 from the Mass General Brigham Biobank, with 44% self-identifying as Black. Using standard FLC reference ranges, 1074 of 10 035 individuals (10.7%) were diagnosed with LC monoclonal gammopathy of undetermined significance (MGUS), with 99% being κ-restricted. In the United States, 14.8% of Black and 4% of White individuals were diagnosed (*P* < .01). Among US participants of African (AFR) and European (EUR) genetic ancestry, 14.4% AFR and 2.9% EUR were diagnosed (*P* < .01). Among South Africans (100% Black), 27.8% were diagnosed using standard ranges. To avoid overdiagnosis, we propose a new κ/λ ratio reference range (0.686 to 2.10) for populations of AFR descent with normal renal function, with standard values for κ and λ being 7.97 to 77.50 mg/L and 6.20 to 49.20 mg/L, respectively. This reduces LC-MGUS overdiagnosis by 91% (10.7% vs 0.97%). Using the new reference, LC-MGUS accounts for 8.8% of MGUS cases, with 74% being κ-restricted, consistent with LC myeloma rates. These findings highlight the importance of basing disease definitions, such as MGUS, on diverse populations. Adopting our proposed FLC reference values would reduce MGUS overdiagnosis among Black individuals, avoiding unnecessary financial, psychological, and medical consequences. This study includes data from NCT03689595.

Introduction

The diagnosis of monoclonal gammopathies relies primarily on detecting a monoclonal component (M protein) in the serum by protein electrophoresis, immunofixation, or mass spectrometry.[1,2] Those techniques fail to detect light chain (LC)–only diseases (κ or λ), which account for ~20% of patients with multiple myeloma (MM).[3] In the past, the diagnosis of LC monoclonal gammopathy of undetermined significance (LC-MGUS) or LC-MM relied on the immunofixation of concentrated urine samples.[4,5] This approach was later updated to measure the concentration of κ and λ free LC (FLC) and the κ/λ FLC ratio (FLCr) in the serum.[6] In 2002, Katzmann et al established reference ranges for serum κ and λ concentrations and FLCr in a cohort of 282 healthy donors from the United States using the Freelite nephelometry assay on a BN II System analyzer.[7]

Consequently, individuals with undetectable M-protein and abnormal FLC values are diagnosed with LC-MGUS. Since 2008, the International Myeloma Working Group (IMWG) recommended using these reference ranges to diagnose LC-MGUS and to assess stringent complete response in MM and hematologic response in amyloid LC amyloidosis.[6,8] The IMWG guidelines' authors and others noted the assay's variability and significant lot-to-lot variation, highlighting the need for caution regarding reference intervals.[6,9,10] This variation relates to the use of different commercial methods (eg, Freelite, Siemens N-Latex, and Sebia FLC Seralite Abingdon Health) and analyzers (eg, BN II, SPA, and Optilite), which could have specific reference ranges.[11-14]

In addition to LC-MGUS, impaired renal filtration in kidney disease causes an increase in λ LC concentration and an even higher increase in κ LC concentration, leading to an elevated FLCr.[15] To limit overdiagnosis of LC-MGUS, "renal reference ranges" have been defined for FLC in individuals with impaired renal function.[16,17] In 2022, the iStopMM study defined new reference ranges based on screening 75 422 healthy individuals aged >40 years in Iceland. After identification of individuals with immunoglobulin A (IgA), IgM, and IgG (heavy chain [HC]) MGUS by serum protein electrophoresis and immunofixation, they defined κ, λ, and FLCr ranges in individuals with different degrees of impaired renal function (estimated glomerular filtration rate [eGFR] of 45-60, 30-44, or <30), reducing overdiagnosis of LC-MGUS in those with renal impairment.[17]

In our recent effort to screen for MGUS in a racially diverse US-based population at risk of developing myeloma, we found that the FLCr was higher in Black individuals than White individuals. This difference remained significant when we considered only individuals with normal renal function.[18] This resulted in an MGUS diagnosis for 26% of Black individuals, inconsistent with known rates of progression and frequency of LC disease in overt MM.[18]

To confirm our initial findings, extend them to an African (AFR) population, and identify clinically useful ranges for Black individuals, we assembled a racially diverse cohort of 10 035 individuals from 3 prospective cohort studies: the PROMISE US study, PROMISE South Africa (ZA), and the Mass General Brigham Biobank (MGBB) in the United States. We screened their serum for monoclonal gammopathies and used negative controls with normal renal function to establish new reference ranges for FLC and FLCr for a racially diverse population, including Black individuals and individuals with AFR ancestry. These new reference ranges will guide the interpretation of LC values and reduce overdiagnosis of MGUS and its downstream consequences in many healthy individuals.

Methods

Population enrolled and screening tests

Individuals included in this study were aged ≥18 years and enrolled on the PROMISE US (ClinicalTrials.gov identifier: NCT03689595, Dana-Farber/Harvard Cancer Center institutional review board number 18-370), or the MGBB (protocol 2021P001703). In the PROMISE ZA cohort, individuals could enroll if they were aged between 40 and 75 years (Dana-Farber/Harvard Cancer Center institutional review board number 21-127, M240565). For the PROMISE US study, eligibility criteria were having a family member with a history of blood cancer or self-reporting as Black.[18,19] Individuals from the PROMISE US study consented to study between 26 February 2019 and 4 June 2024 (n = 8221). PROMISE ZA participants enrolled were screened in the Soweto region of ZA between 5 May 2021 and 22 August 2023 (n = 2020). MGBB's participants included in our study were initially recruited between 28 July 2010 and 1 July 2021, and their banked serum was screened retrospectively. Individuals with an MM diagnosis before the serum draw date were excluded.[18]

Blood was collected in 8.5-mL serum separating tubes; samples from PROMISE US were shipped overnight, processed in a centralized laboratory, and stored at −80°C. Samples from MGBB and PROMISE ZA were directly processed to extract serum and banked at −80°C or shipped to the central laboratory at Dana-Farber Cancer Institute. Upon sample thawing, FLC levels were measured with the Optilite analyzer using the Freelite assay (Binding Site Group). Samples were tested from 29 December 2021 to 10 April 2023 (644 days).

The eGFR and a health questionnaire were used to assess renal function at the time of screening for monoclonal gammopathies. eGFR was estimated upon measurement of creatine or cystatin C (Optilite) and computed with the Chronic Kidney Disease (CKD) Epidemiology Collaboration 2021 Equation,[20] or individuals with creatinine values and CKD Epidemiology Collaboration cystatin C Equation (2012).[21] Impaired renal function was defined by eGFR of <60 or the presence of CKD as self-reported in health-related questionnaires.

Race and genetic ancestry annotation

Race was self-selected from categories Black, White, Asian, Asian/Pacific Islander, Multiracial, or Native American via a questionnaire on the PROMISE study, and from MGBB electronic records. Ethnicity was similarly self-selected from Hispanic or non-Hispanic categories. We grouped all non-Black and non-White participants into the "other" group (Asian, Asian/Pacific Islander, Multiracial, or Native American).

The genetic ancestry of individuals in the MGBB was estimated with DNA genotype array data, imputed using the 1000 Genome (1KG) reference panel.[22] Variants with imputation quality (R^2) of <0.5 were filtered using BCFtools.[23] Additionally, variants with high linkage disequilibrium were pruned based on the following parameters: window size of 50 base pairs, step size of 5 variants, and pairwise r^2 threshold of 0.2. Then, those with minor allele frequencies of <1% were filtered using PLINK.[24] Genetic ancestry was inferred based on clustering using principal component analysis to identify probability for 5 ancestries (superpopulations from the 1000 Genomes Project)[22]: AFR, Admixed American native, South Asian, EUR, and East Asian. We merged the MGBB genotype data with superpopulation-labeled 1KG genotypes, and a total of 3 088 867 overlapping single-nucleotide polymorphisms were used for ancestry estimation. K-nearest neighbor classifier (k = 6) was evaluated using 10 cross-validations and achieved 99.7% accuracy.[24]

Clinical comorbidities annotation for main cardiovascular, diabetes, and rheumatic disorders for individuals in the MGBB biobank were extracted and identified using Phecodes and hierarchical groupings of International Classification of Diseases, ninth and tenth version codes.[18] HIV infection was self-reported in the PROMISE USA and ZA questionnaire.

HC-MGUS was defined by an M protein concentration of >0.2 g/L detected by matrix-assisted laser desorption ionization–time of flight mass spectrometry. The EXENT-iQ software was used to quantify the concentration of M proteins.[25]

Statistical methods

Quantitative variables were described with their median and 95% confidence intervals (95% CIs) or, when specified, with their interquartile range. Groups were compared with the Kruskal-Wallis test and post-hoc pairwise comparisons with the Dunn test, adjusted for multiple testing (Holm false discovery rate procedure). For qualitative variables, the frequency of each modality was described. Groups were compared using the Fisher exact test. Logistic regression models estimated the odds ratio (OR) and 95% CIs for associations with FLC values.

To define a new standard reference range for FLCr, we first established the reference intervals for κ and λ values (95% central values) following Clinical and Laboratory Standards Institute guidelines.[26] Then, using only individuals with both κ and λ within the new reference intervals (removing outlier values), we define the new reference interval for FLCr (95% central values). Finally, individuals were diagnosed with LC-MGUS if they had an FLCr outside the reference range and abnormal involved LC (κ when FLCr was greater than the upper bound of the reference range; λ otherwise). Individuals with impaired renal function were excluded from the group of patients used to define the new reference ranges. For these individuals with impaired renal function, we used the renal ranges for κ, λ and FLCr reported by the iStopMM study.[16,17]

Results

Study participants

We analyzed serum from 10 035 individuals who were screened for MGUS from the PROMISE US, PROMISE ZA, and MGBB studies. Of those, 1007 (10%) screened positive for IgG, IgA, or IgM HC-MGUS and were excluded from subsequent analyses.[18] The remaining cohort comprised 9028 individuals. Within this group, both race and FLC measurements were available for 8975 individuals, including 4826 (54%) from the MGBB and 4149 (46%) from the PROMISE study, of which 2383 (57%) originate from the United States and 1766 (43%) from ZA (Figure 1).

Our study cohort included 3889 (44%) Black individuals (118 [5%] in PROMISE US, 1766 [100%] in PROMISE ZA, and 2005 [42%] in MGBB; Table 1). Females were overrepresented in the PROMISE US program (74%) and MGBB (64%) compared with PROMISE ZA (49%). The PROMISE US population was slightly older (PROMISE US: median aged, 54 years; MGBB: median age, 54 years; PROMISE ZA: median age, 51 years). Both variables were accounted for in our models. Renal function was estimated with eGFR in 3519 participants from MGBB (72%), 103 (4%) from PROMISE US, and 568 (32%) from PROMISE ZA. As expected from a hospital-based prospective study compared with a community healthy population screening, impaired renal function defined by eGFR of <60 and/or self-reported CKD were higher in the MGBB biobank population than in PROMISE US and ZA (MGBB: 432, 9%; PROMISE US: 31, 1.3%; ZA: 8, 0.5%; $P < .001$). Few individuals self-reported Hispanic ethnicity (1.1%), so ethnicity was not included in this analysis.

Genetic ancestry was estimated using DNA genotyping, with data available for 4023 patients in the MGBB study (83% of the screened). No genotyping data were available for the PROMISE US or ZA studies. A total of 1540 (38%) participants in the MGBB genotyping cohort were from AFR superpopulation, 2332 (58%) from the EUR superpopulation, and 151 (4%) were of other ancestries (Admixed American, South Asian, East Asian; Table 1; "Methods").

FLC values and standard reference ranges

In the population with normal renal function and race information available (n = 7225, 80%; 3163 Black, 3894 White, and 140 Other; Figure 1), the FLCr was higher in Black individuals (median, 1.42; 95% CI, 0.84-2.47) than in White individuals (median, 1.17; 95% CI, 0.59-1.96; q = 1.89e−212) or individuals of other races (median, 1.21; 95% CI, 0.68-2.24; q = 4.57e−12; Figure 2A; supplemental Table 1, available on the *Blood* website). As expected, the FLCr was also higher in individuals with impaired renal function (CKD; median, 1.5; 95% CI, 0.82-2.77) than in individuals with normal renal function within race groups (CKD vs Black q = 3.18e−03, CKD vs White q = 1.20e−05, CKD vs Other q = 6.60e−15; Figure 2A; supplemental Table 1). The FLCr was also higher in Black South Africans (median, 1.46; 95% CI, 0.84-2.59) and Black Americans (median, 1.38; 95% CI, 0.85-2.29; q = 1.57e−05) than in White Americans (median, 1.17; 95% CI, 0.59-1.96; q = 1.92e−177) and others (median, 1.21; 95% CI, 0.68-2.24; q = 4.39e−14; Figure 2B; supplemental Table 2). The FLCr were higher in the AFR superpopulation (median, 1.37; 95% CI, 0.88-2.3) than in the EUR (median, 1.15; 95% CI, 0.71-1.91; q = 3.71e−65) or other superpopulations (median, 1.21; 95% CI, 0.76-2.04; q = 1.72e−06; Figure 2C; supplemental Table 3).

FLC κ and λ values were also higher in Black individuals than White individuals and other races (supplemental Results; supplemental Table 1; supplemental Figure 1A-B). Similarly, κ and λ were also higher in Black South Africans than Black Americans and White Americans (supplemental Results; supplemental Table 2; supplemental Figure 1C-D) as well as in the comparison of individuals with AFR compared with EUR ancestry (supplemental Results; supplemental Table 2; supplemental Figure 1E-F)

Using these reference ranges and the renal reference ranges for those with impaired renal function, 1074 individuals of 10 035 (10.7%) would screen positive for LC-MGUS, with 99% of those cases being κ LC-MGUS. Similarly, in the population with normal renal function, 958 individuals (11.9%) would be positive for LC-MGUS (99% of those being κ-restricted). In addition, in the US-based cohort, a total of 351 Black individuals (14.8% of Black Americans) would screen positive for LC-MGUS and 178

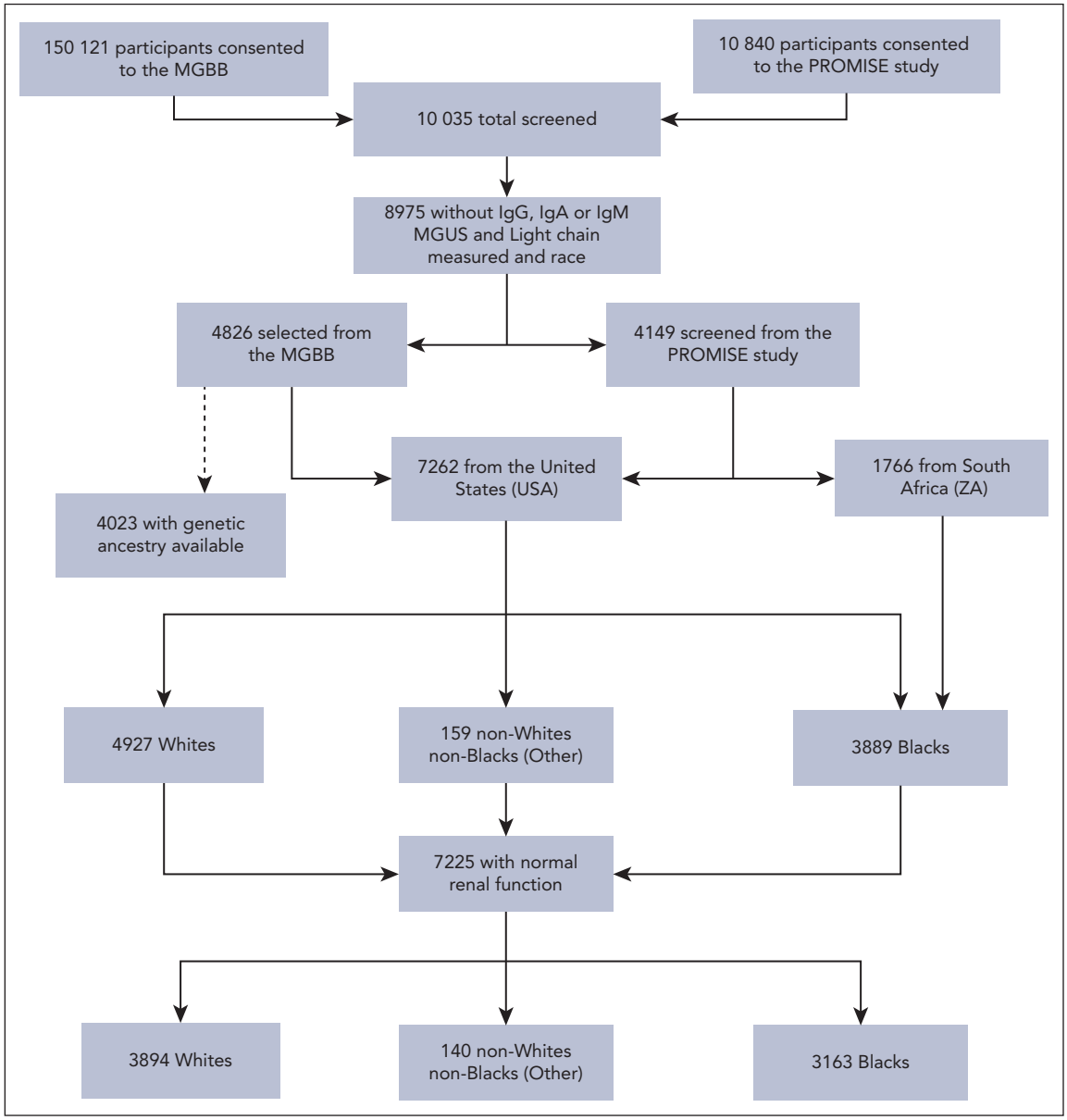

Figure 1. CONSORT diagram of the cohort screened for monoclonal gammopathies. Detailed study cohorts are presented in "Methods."

White individuals (4% of White Americans, q = 8.34e−29). This difference was consistent in the population with normal renal function with higher rates of LC-MGUS in Black individuals (n = 258, 16.5% of Black Americans) than in White (n = 130, 3% of the White Americans; q = 1.02e−21).

A similar difference emerges when comparing US-based participants of AFR genetic ancestry groups (AFR, n = 248/1728 [14.4%]; EUR, n = 76/2608 [2.9%]; q = 6.62e−06) and confirmed in individuals with normal renal function (AFR, n = 185/1118 [16.5%]; EUR, n = 61/1645 [3.7%]; q = 2.26e−04). Using standard reference ranges in a ZA-based cohort, 559 participants (27.8%) would have been diagnosed with LC-MGUS, of which 99.5% were κ (558 individuals with normal renal function).

We then modeled FLCr values as a dependent variable in a logistic regression on self-reported race, age, sex, and eGFR. Black race was associated with a higher FLCr and a predictor of an abnormal FLCr (OR, 1.29; 95% CI, 1.23-1.36; $P < .001$; Table 2). In a multivariate model including information on the country of origin (ZA or US), being Black South African or Black American were independent factors for higher FLCr (OR, 1.37; 95% CI, 1.26-1.48; $P < .001$; and OR, 1.27; 95% CI, 1.21-1.34; $P < .001$; respectively; supplemental Table 4). Similarly, being of AFR ancestry was independently associated with higher FLCr in a model based on genetic ancestry (OR, 1.26; 95% CI, 1.18-1.35; $P < .001$; supplemental Table 5).

Additionally, the probability of being of AFR ancestry was associated with higher rates of abnormal FLCr when we used the Katzmann reference ranges, and this was true across age classes (Figure 2D) as well as in individuals with impaired renal function measured by eGFR (Figure 2E).

Table 1. Demographics of the individuals without an IgG, IgA, or IgM MGUS included in the analysis from 3 cohorts

Study	MGBB	PROMISE US	PROMISE ZA	Total
n	4826 (54%)	2383 (27%)	1766 (20%)	8975 (100%)
Female, n (%)	3086 (63.9%)	1759 (73.8%)	868 (49.2%)	5713 (63.7%)
Age (y), median (IQR)	54 (41-63)	58.3 (50.2-64.5)	51 (46-58)	54 (45-62.8)
Race Black, n (%)	2005 (42%)	118 (5%)	1766 (100%)	3889 (44%)
Ethnicity Hispanic, n (%)	47 (1%)	54 (2%)	0 (0%)	101 (1%)
CKD, n (%)	432 (12.3%)	31 (1.3%)	8 (0.5%)	471 (6.1%)
eGFR evaluable, n (%)	3519 (72.9%)	103 (4.3%)	568 (32.2%)	4190 (46.7%)
eGFR, median (IQR)	88 (72.7-101.8)	109.2 (90.5-119.5)	118.2 (105.6-133.5)	92.2 (75.6-107)
eGFR of <60, n (%)	432 (9.0%)	5 (0.2%)	7 (0.4%)	444 (4.9%)
Genetic ancestry evaluable, n (%)	4023 (83%)	0 (0%)	0 (0%)	4023 (45%)
AFR ancestry, n (%)	1540 (38%)	0 (0%)	0 (0%)	1540 (38%)
EUR ancestry, n (%)	2332 (58%)	0 (0%)	0 (0%)	2332 (58%)
Other ancestry, n (%)	151 (4%)	0 (0%)	0 (0%)	151 (4%)

IQR, interquartile range.

Finally, we investigated the association between FLCr and medical conditions reported in the screening study (supplemental Table 6). We found an association in univariate logistic regression analysis between FLCr and cardiac heart failure, diabetes, diabetes complications, and HIV infection status (supplemental Table 7). However, when accounting for sex, age, race, and eGFR in a multivariate regression analysis, none of these variables (cardiac heart failure, diabetes, diabetes complications, and HIV) remained significant (supplemental Table 8). Therefore, we conclude that age, sex, race, and eGFR likely drive the increased FLCr in this model.

The development of a new reference range based on race and ancestry

Taken together, these data emphasized the need for new reference ranges that take into consideration racial diversity in individuals with normal renal function. To that aim, we identified individuals with eGFR of >60 or with no renal impairment, which included 3163 Black individuals (35%), 3894 White individuals (43%), and 140 individuals of other races (2%; Figure 1). Reference intervals for κ were between 7.97 and 77.50 mg/L; reference intervals for λ were between 6.2 and 49.2 mg/L. To define the reference interval of FLCr, we analyzed the FLCr values of a population with both κ and λ inside the reference. In this group of 6449 individuals, the new reference range for FLCr was 0.686 to 2.10.

Applying the renal reference ranges to individuals with impaired renal function,[17] and our new reference range to individuals with normal renal function, 97 individuals of 10 035 (0.97%) would be defined as LC-MGUS. Of these, 25 individuals (26%) would have an abnormal λ and 72 individuals an abnormal κ (74%) LC. Among all individuals diagnosed with MGUS (n = 1104), 91.2% were classified as HC-MGUS and 8.7% LC-MGUS.

In the US cohort, rates of LC-MGUS in the overall screened individuals decreased from 14.8% to 0.8% in Black individuals (n = 18/2375) and from 2.8% to 0.4% in White individuals (n = 21/5417; q = 0.012), comparable with previously reported frequencies.[27] This reduction was similar in the population with normal renal function (Black, n = 11/1559 [0.7%]; White, n = 20/4286 [0.5%]; q = 0.080; Figure 3A-B).[27]

Furthermore, in the AFR population, LC-MGUS decreased from 14.4% to 0.7% (n = 12/1728) and from 2.9% to 0.5% in the EUR population (n = 12/2608; q = 0.003). This was confirmed in individuals with normal renal function (AFR, n = 5/1118 [0.4%]; EUR, n = 61/1645 [0.7%]; q = 0.06; Figure 3C).

Using new reference ranges in a ZA-based cohort, 56 participants (2.8%) would have been diagnosed with LC-MGUS compared with 27.9%. Additionally, in patients with MGUS with an HC M protein detected (n = 1007), rates of abnormal FLCr decreased from 29.2% to 8.6%, with a reduction from 41% to 11.7% in Black individuals and from 17.6% to 5.3% in White individuals.

Discussion

The detection of LC monoclonal gammopathies relies on comparing FLC values against reference ranges established >20 years ago, primarily in White individuals.[7] Reference ranges must be applicable to the general population to avoid overdiagnosing or underdiagnosing individuals with cancerous or precancerous conditions, which can have significant

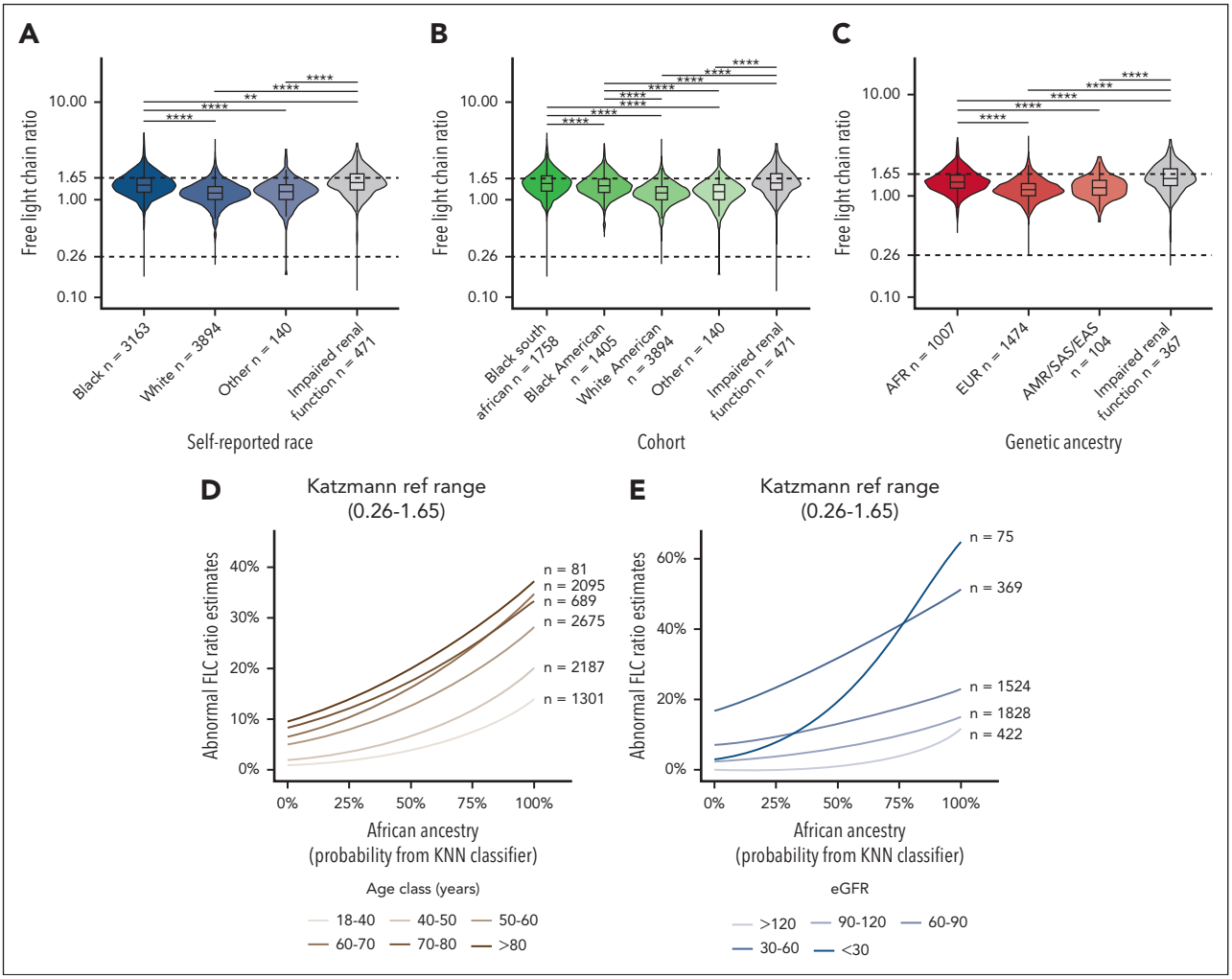

Figure 2. Individuals of Black self-reported race or AFR genetic ancestry with normal renal function have higher κ, λ, and FLCr. (A) FLCr κ/ λ in individuals by race and renal function: Black individuals with normal renal function (n = 3163; 41%), White individuals with normal renal function (n = 3894; 51%), individuals of other races with normal renal function (others; n = 140; 2%) and individuals with impaired renal function (n = 471, 6%); (B) FLCr κ/ λ in individuals by cohort and renal function: Black South Africans (n = 1758, 23%), Black Americans (n = 1405, 18%), White Americans (n = 3894; 51%), Americans of other races (other; n = 140, 2%), and individuals with impaired renal function (n = 471, 6%); (C) FLCr κ/ λ in individuals by genetic ancestry and renal function: Africa (AFR; n = 1007, 34%) , Europe (EUR; n = 1475, 50%), other ancestry (Americas (AMR)/ South Asia (SAS)/East Asia (EAS); n = 104, 4%) and individuals with impaired renal function (n = 367, 12%). The vertical axis is represented in $\log_{10}$ scale. Dashed horizontal lines represent the reference range for the FLCr (0.26-1.65). Bars over violins represent the results of pairwise statistical comparisons (****$P < .0001$; ***$P < .001$; **$P < .01$; *$P < .05$ [adjusted P values]). P values >.05 are not shown. (D-E) Estimates for abnormal FLCr using the Katzmann standard reference range (0.26-1.65) dividing the population by age group and AFR ancestry proportion as a continuous variable (D); and comparing individuals with different ranges of eGFR (E). KNN, k-nearest neighbors.

Table 2. Logistic regression of baseline factors affecting FLCr: self-reported race, age, sex, and renal function

	OR	95% CI	*P* value
Black race (vs White)	1.29	1.23-1.36	4.67e−26
Other race (vs White)	1.09	0.89-1.34	4.08e−01
Age*	1.04	1.02-1.06	2.62e−05
Sex: male	1.02	0.97-1.07	3.90e−01
eGFR†	0.97	0.96-0.98	1.26e−09

*Age by decade.

†eGFR by 10 mL/min.

implications, including strain on the medical system, increased anxiety, and changes in medical coverage and life insurance.

In this study, we demonstrated that FLC values (κ, λ, and the FLCr) are higher in self-reported Black individuals. This increase was not because of higher rates of MGUS in Black populations,[28] because IgG, IgA, and IgM MGUS were excluded, because of impaired renal function, or because we selected individuals with normal renal function and adjusted our models for eGFR. We confirmed this FLC difference in 2 independent cohorts, including Black individuals in the US and ZA. A previous study in ZA did not report increased FLC values in a healthy population of 120 individuals who screened negative for MGUS with protein electrophoresis. However, this cohort was young (median age, 37 years [range, 22-60]), and only 50% were Black, so the signal

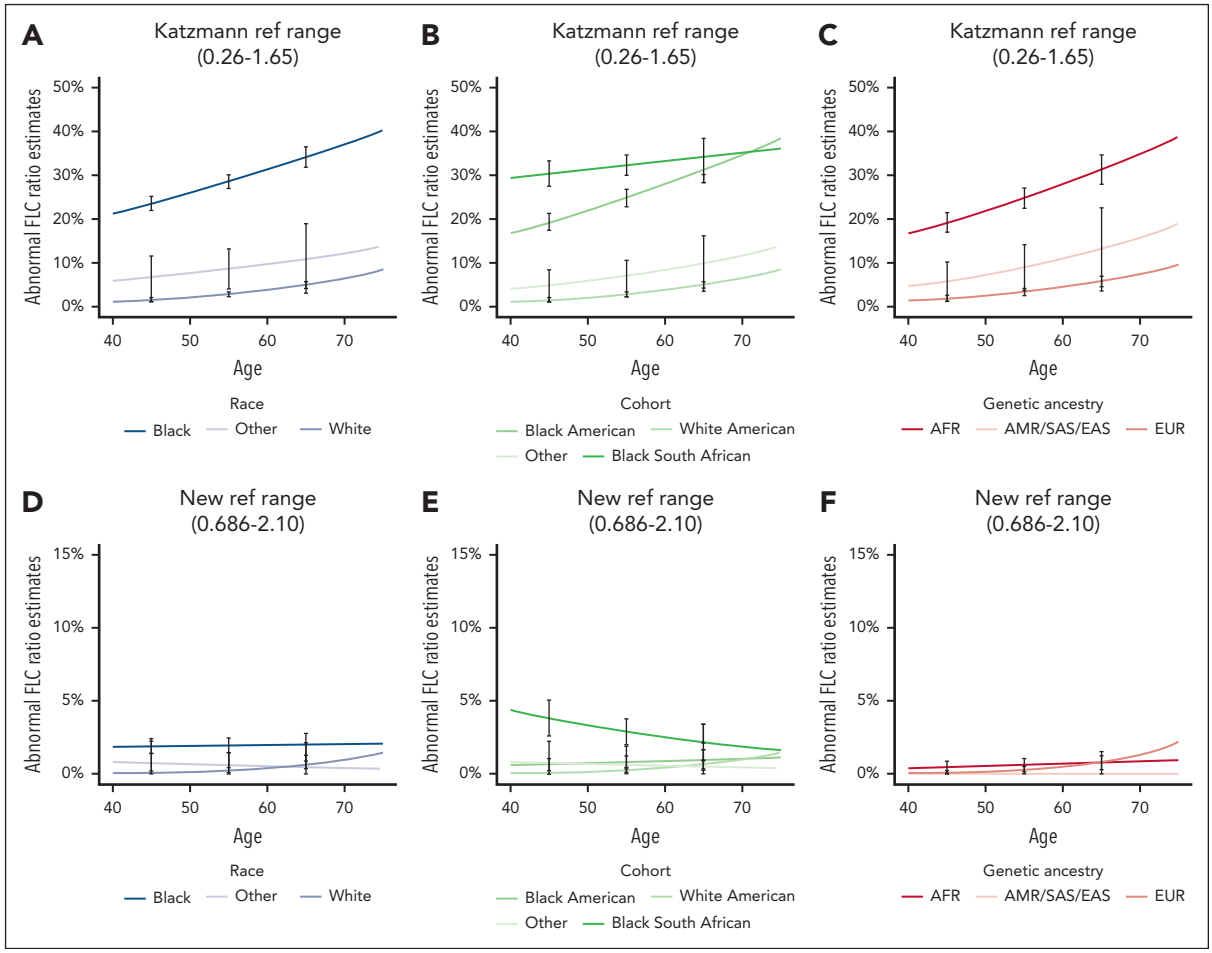

Figure 3. Abnormal free light chain ratio and abnormal involved light chain according to standard reference range and new reference for a diverse population. (A-C) Estimates for abnormal FLCr using the Katzmann standard reference (ref) range (0.26-1.65) analyzing the population by race (A), cohort (B; Black American, White American, Black South African, other), and genetic ancestry (C; AFR, EUR, and other [AMR/SAS/EAS]). (D-F) Estimates for abnormal FLCr using a new ref range (0.686-2.10) analyzing the population by race (D), cohort (E; Black American, White American, Black South African, and other), and genetic ancestry (F; AFR, EUR, and other [AMR/SAS/EAS]).

may not have been detected.[29] To our knowledge, higher levels of FLC in Black individuals have not been reported previously.

Our study also observed, to a lesser extent, higher FLC values in Whites compared with previous studies. This finding justified redefining FLC standard ranges when using the Freelite assay with an Optilite analyzer because of a phenomenon known as κ drift.[30-32] For example, Treger et al performed a large retrospective epidemiology study in 5075 individuals who were negative for MGUS tested by immunofixation and found that 25% had an FLCr outside the reference range. Treger et al defined a new reference range of 0.67 to 2.13, similar to our reference range but with a lower threshold for abnormal κ (60.5 vs 77.5 mg/L) and similar for λ (49.2 vs 46.6 mg/L).[33]

We acknowledge that our results and reference ranges may apply to Freelite- and Optilite-based tests[13,34]; however, comparative studies are needed for other methods to either corroborate our findings or establish specific reference ranges for each FLC assay and analyzer.[11] In fact, the study by Treger et al used different instruments and spanned 10 years, which may be associated with the observed κ drift. In addition, in Treger et al's study, FLC tests were requested in the clinical setting, contrasting with the presumably healthy community-based individuals in the PROMISE ZA study. These factors may explain why the authors found a similar FLC range, despite only 4.5% of participants in their study identifying as Black, compared with 44% in this study.[33]

In individuals with reduced eGFR, reference range for κ, λ, and FLCr would lead to overdiagnosis of LC monoclonal gammopathy. Recently, the Icelandic screening study for MGUS (iStopMM) redefined 95% CI for κ, λ, and FLCr in individuals with decreasing eGFR rates, allowing for better identification and reducing overdiagnosis in those populations.[17] Our new reference range for a diverse population with normal renal function (FLCr between 0.686 and 2.10) results in an LC-MGUS diagnosis rate of 0.94%, comparable with the 0.5% to 1.3% reported in the initial Olmsted County study.[27] The proportion of HC- vs LC-MGUS in our study aligns with that of patients with newly diagnosed myeloma,[3] and screened smoldering multiple myeloma (SMM) population in the iStopMM study.[35] Furthermore, the κ vs λ isotype rates of LC-MGUS in our study are comparable with those in patients with newly diagnosed LC-MM.[3]

The use of FLC is not limited to diagnosing LC-MGUS, so the implications of our work for other plasma cell dyscrasias should be explored in the future. FLCr is included in most risk stratification

scores for SMM, such as the IMWG and the Mayo 20-2-20 model,[36,37] as well as MGUS risk stratification models and in SLiM criteria for progression from SMM to MM.[38,39] Importantly, applying our new reference range to individuals with HC-MGUS decreases the rates of abnormal FLCr from 29% to 5%, affecting their risk categorization and potentially reducing the need for further testing, such as bone marrow biopsy. Additionally, there is limited knowledge regarding the prediction of progression from LC-MGUS or SMM to MM, particularly in Black individuals.[27,40] Therefore, we believe the first step is to accurately identify individuals with LC-MGUS or SMM, minimizing overdiagnosis and refining the definitions of clinical progression.

FLC is included in assessing the depth of response to therapy in patients with MM (stringent complete response) and amyloid LC amyloidosis. Confirming the impact of an optimal reference range is crucial for response assessment across different racial backgrounds and will be the next step of our research.[8]

This supports the use of our new reference range until newer techniques, such as mass spectrometry, become available to diagnose LC-only disease.[41] An important next step would be to use mass spectrometry to estimate the false-positive and false-negative rates of this new range.

This study did not investigate potential biological mechanisms behind the differences in FLC concentrations between Black and White individuals. The increase in abnormal FLCr with AFR genetic ancestry, independent of kidney function, and age, suggests an underlying genetic variability rather than environmental or other external factors. In a preliminary analysis including clinical conditions (cardiovascular comorbidities, diabetes, and HIV), we did not find a significant association with FLC when adjusting for renal function, age, and race. In the future, we will collaborate with other groups to examine candidate germ line variants that correlate with differences in plasma cell secretion or renal tubular reabsorption of LCs. This could also help in better understanding the underlying increased incidence of monoclonal gammopathies in Black individuals and potentially therapeutic strategies.

In summary, we provide evidence of a skewed κ-to-λ ratio in individuals of Black race or with AFR genetic ancestry, both from the United States and ZA. We showed that race and renal function independently affect FLCr. We defined reference ranges using a racially diverse population with normal renal function. To better represent our current global diversity, we strongly believe that the definitions of disease entities (eg, MGUS) should be based on diverse populations before applying them globally. Differences in standard biomarker levels are known among populations with different ancestries, such as white blood cell counts in benign ethnic neutropenia (explained by Duffy-null allele) and hemoglobin A1c levels for diabetes.[42,43] Similarly, FLC reference levels should be adjusted to represent Black individuals better. For the same reason, more studies are warranted to examine the impact of FLC levels on IMWG response criteria and SLiM-CRAB criteria in Black patients.

Acknowledgments

The authors thank all study participants who contributed to this study. The authors also thank Anna V. Justis, a medical writer employed by Dana-Farber Cancer Institute, who edited the manuscript. The authors thank the Mass General Brigham Biobank for providing samples and health information data used in this study.

This work was supported in part by the National Institutes of Health, National Cancer Institute (grants R35CA263817 and U01CA271492 awarded to I.M.G.) and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation. This research was also supported by a Stand Up To Cancer Dream Team Research grant (SU2C-AACR-DT-28-18). Stand Up To Cancer is a program of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the scientific partner of Stand Up To Cancer. Opinions, interpretations, conclusions, and recommendations are those of the author(s) and are not necessarily endorsed by Stand Up To Cancer, the Entertainment Industry Foundation, or the American Association for Cancer Research.

Authorship

Contribution: L.B., J.-B.A., H.E.-K., D.J.L., M.J., W.C.C., and I.M.G. conceptualized the study; L.B., J.-B.A., D.J.L., H.E.-K., S.K., L.T., C.R.M., G.G., M.J., W.C.C., and I.M.G. contributed to the study methodology; L.B., J.-B.A., H.E.-K., D.J.L., S.K., G.F., C.M., S.A., E.D.L., J.C., M.I.D., J.P., L.N.G., V.P., N.S., D.S., M.P., S.H., D.T., E.W.K., C.R.M., M.J., W.C.C., and I.M.G. were responsible for study investigation; J.-B.A., L.B., M.J., W.C.C., and I.M.G. were responsible for data visualization; M.J., W.C.C., and I.M.G. were responsible for funding acquisition; M.J., W.C.C., and I.M.G. supervised the study; L.B., J.-B.A., M.J., W.C.C., and I.M.G. wrote the original manuscript draft; and all authors contributed to discussions of content and reviewed and edited the manuscript before submission.

Conflict-of-interest disclosure: I.M.G. is a consultant for GlaxoSmithKline, Sanofi, Janssen, Takeda, Karyopharm, AbbVie, Gene Network Sciences, Cellectar, Medscape, Menarini Silicon Biosystems, Genentech, Adaptive, Bristol Myers Squibb, Aptitude, Curio Science, and Oncopeptides; has received honoraria from Aptitude; and is a founder of, and holds privately held equity in, Predicta Biosciences. C.R.M. serves on the Exact Sciences Corporation advisory board and steering committee for Natera, Inc and receives honoraria and travel support from Exact Sciences. D.S., M.P., S.H., and D.T. are employees of the Binding Site, part of Thermo Fisher Scientific. G.G. receives research funds from International Business Machines Corporation, Pharmacyclics/AbbVie, Bayer, Genentech, Calico, and Ultima Genomics; is an inventor on patent applications filed by the Broad Institute related to MSMuTect, MSMutSig, PolySolver, SignatureAnalyzer-GPU, MSEye, and MinumuMM-seq; is a founder, consultant, and holds privately held equity in Scorpion Therapeutics; and is a founder of and holds privately held equity in Predicta Biosciences. The remaining authors declare no competing financial interests.

ORCID profiles: L.B., 0000-0003-3722-9696; J.-B.A., 0000-0002-4218-7294; H.E.-K., 0000-0002-4023-6566; S.K., 0000-0003-4815-0799; J.C., 0009-0007-6268-7038; V.P., 0009-0008-5503-9291; D.S., 0000-0001-8430-0120; C.R.M., 0000-0002-9128-3012; W.C.C., 0000-0002-3248-4906; M.J., 0000-0001-8616-9672; I.M.G., 0000-0001-7361-3092.

Correspondence: Irene M. Ghobrial, Dana-Farber Cancer Institute, 450 Brookline Ave, LC8116, Boston, MA 02215; email: irene_ghobrial@dfci.harvard.edu.

Footnotes

Submitted 9 July 2024; accepted 30 October 2024; prepublished online on *Blood* First Edition 21 November 2024. https://doi.org/10.1182/blood.2024026078.

*L.B. and J.-B.A. are joint first authors.

†W.C.C., M.J., and I.M.G. are joint senior authors.

Data from the PROMISE study can be made available in aggregate after deidentification to investigators who submit appropriate proposals to the study team (PROMISE, NCT03689595). Inquiries should be directed to the corresponding author, Irene M. Ghobrial (irene_ghobrial@dfci.harvard.edu).

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

REFERENCES

1. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538-e548.
2. Murray DL, Puig N, Kristinsson S, et al. Mass spectrometry for the evaluation of monoclonal proteins in multiple myeloma and related disorders: an International Myeloma Working Group Mass Spectrometry Committee Report. *Blood Cancer J*. 2021;11(2):24.
3. Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc*. 2003;78(1):21-33.
4. Shaheen SP, Levinson SS. Serum free light chain analysis may miss monoclonal light chains that urine immunofixation electrophoreses would detect. *Clin Chim Acta*. 2009;406(1-2):162-166.
5. Kyle RA. Sequence of testing for monoclonal gammopathies serum and urine assays. *Arch Pathol Lab Med*. 1999;123(2):114-118.
6. Dispenzieri A, Kyle R, Merlini G, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia*. 2009;23(2):215-224.
7. Katzmann JA, Clark RJ, Abraham RS, et al. Serum reference intervals and diagnostic ranges for free κ and free λ immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem*. 2002;48(9):1437-1444.
8. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016;17(8):e328-e346.
9. Tate JR, Gill D, Cobcroft R, Hickman PE. Practical considerations for the measurement of free light chains in serum. *Clin Chem*. 2003;49(8):1252-1257.
10. Pattenden RJ, Rogers SY, Wenham PR. Serum free light chains; the need to establish local reference intervals. *Ann Clin Biochem*. 2007;44(Pt 6):512-515.
11. Cotten SW, Shajani-Yi Z, Cervinski MA, Voorhees T, Tuchman SA, Korpi-Steiner N. Reference intervals and diagnostic ranges for serum free κ and free λ immunoglobulin light chains vary by instrument platform: implications for classification of patient results in a multi-center study. *Clin Biochem*. 2018;58:100-107.
12. White-Al Habeeb NMA, Earle T, Spencer M, Blasutig IM. Evaluation of the N-latex serum free light chain assay on the Siemens BNII analyzer and agreement with the Binding Site FreeLite assay on the SPAPlus. *Clin Biochem*. 2018;51:90-96.
13. Fleming CKA, Swarttouw T, De Angelino CMK, Jacobs JFM, Russcher H. Method comparison of four clinically available assays for serum free light chain analysis. *Clin Chem Lab Med*. 2020;58(1):85-94.
14. Hoedemakers RMJ, Pruijt JFM, Hol S, et al. Clinical comparison of new monoclonal antibody-based nephelometric assays for free light chain kappa and lambda to polyclonal antibody-based assays and immunofixation electrophoresis. *Clin Chem Lab Med*. 2011;50(3):489-495.
15. Hutchison CA, Basnayake K, Cockwell P. Serum free light chain assessment in monoclonal gammopathy and kidney disease. *Nat Rev Nephrol*. 2009;5(11):621-628.
16. Hutchison CA, Harding S, Hewins P, et al. Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. *Clin J Am Soc Nephrol*. 2008;3(6):1684-1690.
17. Long TE, Indridason OS, Palsson R, et al. Defining new reference intervals for serum free light chains in individuals with chronic kidney disease: results of the iStopMM study. *Blood Cancer J*. 2022;12(9):133.
18. El-Khoury H, Lee DJ, Alberge J-B, et al. Prevalence of monoclonal gammopathies and clinical outcomes in a high-risk US population screened by mass spectrometry: a multicentre cohort study. *Lancet Haematol*. 2022;9(5):e340-e349.
19. Lee DJ, El-Khoury H, Tramontano AC, et al. Mass spectrometry-detected MGUS is associated with obesity and other novel modifiable risk factors in a high-risk population. *Blood Adv*. 2024;8(7):1737-1746.
20. Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin C–based equations to estimate GFR without race. *N Engl J Med*. 2021;385(19):1737-1749.
21. Shlipak MG, Matsushita K, Ärnlöv J, et al. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med*. 2013;369(10):932-943.
22. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74.
23. Li H, Handsaker B, Wysoker A, et al. The sequence alignment/map format and SAMtools. *Bioinformatics*. 2009;25(16):2078-2079.
24. Pedregosa F, Michel V, Grisel O, et al. Scikit-learn: machine learning in Python. *J Mach Learn Res*. 2011;12:2825-2830.
25. Sakrikar D, Marrot N, North S, et al. Multi-site verification of the automated EXENT(R) MALDI-TOF-MS system and immunoglobulin isotypes assay for the identification and quantification of monoclonal immunoglobulins. Paper presented at: AACC Annual Scientific Meeting; 28 September 2021; Atlanta, GA. Accessed 7 October 2021. https://www.abstractsonline.com/pp8/#!/9313/presentation/23
26. Horowitz Gary L, Altaie Sousan, Boyd James C, et al. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline*. 3rd ed. Clinical and Laboratory Standards Institute; 2010.
27. Dispenzieri A, Katzmann JA, Kyle RA, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. *Lancet*. 2010;375(9727):1721-1728.
28. Marinac CR, Ghobrial IM, Birmann BM, Soiffer J, Rebbeck TR. Dissecting racial disparities in multiple myeloma. *Blood Cancer J*. 2020;10(2):19.
29. Zemlin AE, Rensburg MA, Ipp H, Germishuys JJ, Erasmus RT. Verification of serum reference intervals for free light chains in a local South African population. *J Clin Pathol*. 2013;66(11):992-995.
30. Murray D, Dispenzieri A, Kumar S, et al. Free light chain assay drift: potential for misdiagnosis? *J Appl Lab Med*. 2020;5(6):1411-1413.
31. Rozenova K, Willrich M, Snyder M, et al. Kappa free light chain drift prompts the need for a new upper limit of normal free light chain ratio to avoid an epidemic of kappa light chain monoclonal gammopathy of undermined significance. *J Appl Lab Med*. 2023;8(4):742-750.
32. Griffiths M, Schneider RJ, Kulasingam V. Serum free light chain and drift: calibrator adjustment needed? *J Appl Lab Med*. 2024;9(2):394-396.
33. Treger RS, Mathias PC, Cowan AJ, et al. Data analytics improves the diagnostic accuracy of serum free light chain results for detecting monoclonal gammopathy. *Am J Clin Pathol*. 2024;161(3):216-231.
34. Van Hoovels L, Vercammen M, Nevejan L, et al. Serum free light chain analysis: persisting limitations with new kids on the block. *Clin Chem Lab Med*. 2022;60(9):1440-1448.
35. Thorsteinsdóttir S, Gíslason GK, Aspelund T, et al. Prevalence of smoldering multiple myeloma based on nationwide screening. *Nat Med*. 2023;29(2):467-472.
36. Lakshman A, Rajkumar SV, Buadi FK, et al. Risk stratification of smoldering multiple

myeloma incorporating revised IMWG diagnostic criteria. *Blood Cancer J*. 2018;8(6):59.

37. Mateos MV, Kumar S, Dimopoulos MA, et al. International Myeloma Working Group risk stratification model for smoldering multiple myeloma (SMM). *Blood Cancer J*. 2020;10(10):102.

38. Rajkumar S V, Kyle RA, Therneau TM, et al. Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood*. 2005;106(3):812-817.

39. Landgren O, Hofmann JN, McShane CM, et al. Association of immune marker changes with progression of monoclonal gammopathy of undetermined significance to multiple myeloma. *JAMA Oncol*. 2019;5(9):1293-1301.

40. Kyle RA, Larson DR, Therneau TM, et al. Clinical course of light-chain smouldering multiple myeloma (idiopathic Bence Jones proteinuria): a retrospective cohort study. *Lancet Haematol*. 2014;1(1):e28-e36.

41. Bomsztyk J, Ravichandran S, Giles HV, et al. Complete responses in AL amyloidosis are unequal: the impact of free light chain mass spectrometry in AL amyloidosis. *Blood*. 2024;143(13):1259-1268.

42. Khosla L, Bhat S, Fullington LA, Horlyck-Romanovsky MF. HbA1c performance in African descent populations in the United States with normal glucose tolerance, prediabetes, or diabetes: a scoping review. *Prev Chronic Dis*. 2021;18:200365, 16.

43. Bagheri M, Chung CP, Dickson AL, Van Driest SL, Borinstein SC, Mosley JD. White blood cell ranges and frequency of neutropenia by Duffy genotype status. *Blood Adv*. 2023;7(3):406-409.