Freelite for Measurement of Urine-Free Light Chains in Monoclonal Gammopathies

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Abstract

Monoclonal gammopathies are characterized by production of monoclonal immunoglobulin heavy or light chains. Historically, the standard for measuring monoclonal light chains was indirect, requiring 24-hour urine collection, measurement of total protein, and extrapolation of light chain concentration using electrophoresis. In 2001, Freelite was developed allowing direct detection of serum-free light chains (FLCs) with sensitivity >2-log higher than immunofixation electrophoresis (IFE), and became standard for diagnosis and monitoring of these disorders. However, cases are missed when urine FLCs are not measured. We studied 23 individuals with monoclonal gammopathies to determine whether Freelite could be used to measure urine FLCs from a spot urine. We concurrently measured serum and urine FLCs using Freelite and serum heavy chains. Monoclonal urine and serum FLCs correlated in 39% of patients. Both urine and serum monoclonal FLCs correlated with clonal serum heavy chains in 25%, serum in only 30%, and urine in only 10%. We conclude that measurement of spot urine FLCs using Freelite may replace 24-hour urine total protein and IFE. While serum FLCs are adequate in most patients, we found a small number of patients for whom urine FLCs outperformed serum FLCs.

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Introduction

Monoclonal gammopathies represent a spectrum of clonal plasma cell disorders that form monoclonal gammopathy of undetermined significance (MGUS) through active multiple myeloma (MM) that are characterized by production of monoclonal immunoglobulin heavy and/or light chains.[1-3] In these disorders, detection of free light chains (FLCs) has been an evolving and, at times, critical analysis for diagnosis, prognosis, and management of treatment.[4-8]

Historically, the standard for evaluating monoclonal light chain production has been the measurement of urine excretion of light chains determined by collecting a 24-hour urine specimen, measuring 24-hour urine total protein, and extrapolating the quantity of light chains using data from a urine protein electrophoresis (UPEP) and immunofixation electrophoresis (IFE).[1] In 2001, Freelite was developing the utilization of immunonephelometric technology that allowed for improved detection of serum FLCs.[9] Sensitivity of UPEP is 500 to 2000 mg/L, IFE is 150 to 500 mg/L, and Freelite is 1.5 to 3.0 mg/L.[10] It was then demonstrated that serum Freelite alone detected more plasma cell disorders than older methods,[7] with the higher detection rate likely due to the increased sensitivity and ability to measure the ratio of abnormal to normal light chains.

Serum FLCs utilizing Freelite is now integrated into the standard of care in diagnosis and monitoring of plasma cell disorders. However, this should not replace obtaining urine IFE, as a small number of cases were missed when FLCs were not also measured.[1,6] It has been our practice to use Freelite to measure the quantity of FLCs in single urine samples instead of obtaining a 24-hour urine sample with total protein and IFE. However, this has not been recommended for routine screening due to paucity of data using this assay for urinary measurements, and due to the concern for possibly confounding variable excretion of FLCs because of changes in renal function and variable renal reabsorption and degradation of light chains.[10]

The goal of this study was to evaluate the utility of using Freelite for measurement of spot urine FLCs.

Methods

This study was approved by the Albany VA Medical Center Institutional Review Board. We identified individuals with the diagnosis of MGUS, smoldering MM (SMM), or MM, who had multiple concurrent measurements of serum and urine FLCs using Freelite, and serum heavy chains (quantitative immunoglobulins). Urine and serum FLCs were measured using Freelite (Binding Site Ltd, Birmingham, UK) by a reference laboratory (Lab Corp, Burlington, NC). All analyses were performed using the involved/monoclonal FLC levels and involved/monoclonal heavy chain levels, when affected. Serum immunoglobulins (immunonephelometry) along with other serum and urine

TABLE 1. Patients Age, Diagnosis, Mean Serum Creatinine, Number of Paired Data Points, and Number of Months Between Obtaining First and Last Paired Samples .

Disease Type	Age at Diagnosis	Mean Serum Creatinine mg/dL (N)	Urine/Serum Light Chain Matched Points	Months Between First and Last Paired Samples
IgG κ Active MM	72	1.6 (20)	20	24
IgG κ Active MM*	62	0.9 (7)	7	29
IgG κ Active MM	84	1.1 (6)	6	6
IgG κ Active MM	65	1.2 (18)	18	28
IgG κ Active MM	81	2.1 (8)	8	16
IgG κ Active MM	82	1.1 (6)	6	19
IgG κ Active MM	74	1.0 (13)	13	31
IgG κ Active MM	53	1.3 (8)	8	8
IgG λ Active MM	67	1.3 (13)	14	27
IgG κ Active MM	72	0.8 (3)	3	3
IgG κ Active MM	75	0.7 (5)	5	5
IgG λ Active MM	55	1.0 (19)	20	23
λLC Active MM	68	2.5 (20)	21	30
λLC Active MM	56	1.0 (18)	18	14
IgG κ SMM	81	1.0 (4)	4	6
IgG κ SMM	87	1.0 (4)	4	10
IgG λ SMM +	65	0.8 (6)	6	25
IgA κ SMM	69	1.0 (6)	6	12
IgG κ MGUS	90	0.8 (6)	6	19
IgG λ MGUS	60	1.3 (5)	5	17
IgG λ MGUS	87	1.8 (5)	5	22
IgA κ MGUS	67	1.1 (5)	5	23
IgA κ MGUS	62	1.4 (4)	4	13
Mean ± SD	71 ± 10.6	1.2 ± 0.4	9.2 ± 5.9	17.9 ± 8.4

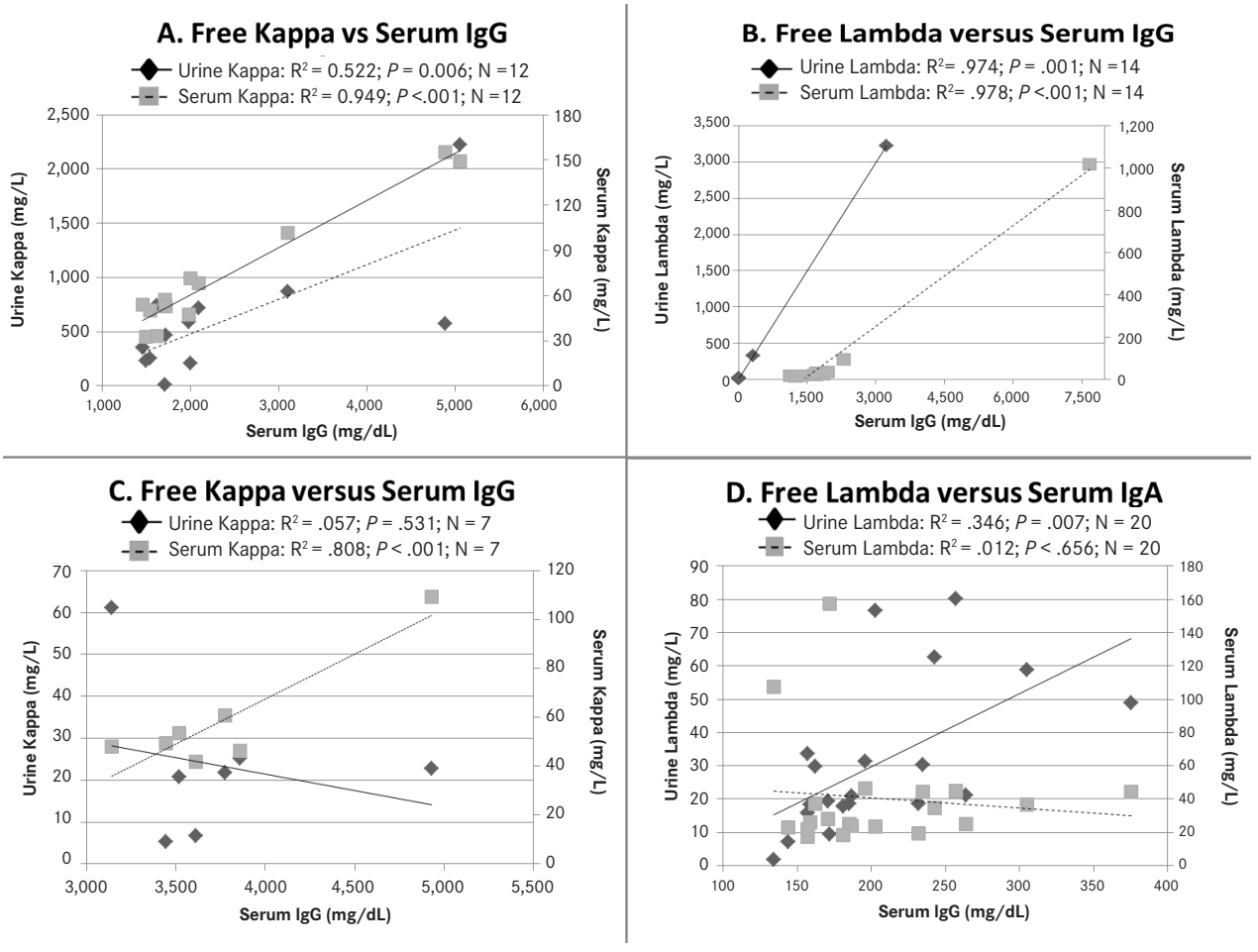
* Prior MGUS.
\+ Insufficient heavy chain data for comparisons.
IgA indicates immunoglobulin A; IgA κ, immunoglobulin A kappa; IgA λ, immunoglobulin A lambda; IgG, immunoglobulin G; IgG κ, immunoglobulin G kappa; IgG λ, immunoglobulin G lambda; λ LC, lambda light chain; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; SD, standard deviation; and SMM, smoldering multiple myeloma.

parameters were measured using standard and widely available techniques by the study's American College of Pathologists accredited medical center chemistry laboratory. Data were analyzed for correlation between parameters using Pearson product-moment correlation. *P* values < .05 were considered significant.[11]

Results

Characteristics of the study population are illustrated in **Table 1**. We identified 23 individuals (all male, consistent with our veteran patient population) with mean (± standard deviation [SD]) age of 71 ± 10.6 years at diagnosis. Five individuals had MGUS, 4 had SMM, and 14 had MM.[12] Two of the 14 patients with MM progressed from MGUS. Twenty-one of 23 patients had intact monoclonal disease, and FLCs were measurable in all 21 of these patients. Two of the 13 patients with MM had light-chain-only disease. A mean (±SD) of 9.2 ± 5.9 matched serum and urine FLC determinations per patient (range, 4–21) was obtained over a mean 17.9 ± 8.4 months. Mean (±SD) serum creatinine of the patients was 1.2 ± 0.4 mg/dL (range, 0.7–2.5).

FIGURE 1. Comparisons Between Abnormal/Monoclonal Urine and Serum FLCs with Abnormal/Monoclonal Serum Heavy Chain. Panels A and B Illustrate 2 Patients for Whom Both Serum and Urine FLCs Correlate with Serum Heavy Chain. Panel C Illustrates a Patient for Whom Only the Serum FLC Correlates with the Serum Heavy Chain. Panel D Illustrates a Patient for Whom Only the Urine FLC Correlates With the Serum Heavy Chain.



FLC indicates free light chain; IgA, immunoglobulin A; and IgG, immunoglobulin G

One individual with SMM was excluded from the heavy chain correlations due to insufficient serum FLC data.

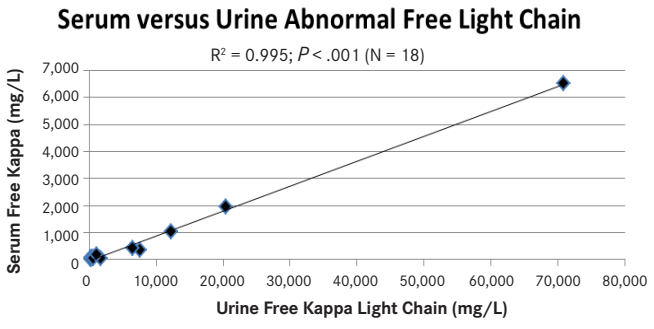
Results of comparisons between immunoglobulin parameters are illustrated in **Table 2**. Overall, the urine FLCs correlated with serum FLCs in 9 (39%) individuals, including both patients with light-chain MM. Both urine and serum FLCs correlated with heavy chain in 5 (25%) patients. Serum FLCs, but not urine FLCs, correlated with serum heavy chain in 6 (30%). Urine FLCs, but not serum FLCs, correlated with heavy chain in 2 (10%). There was no correlation between urine or serum FLCs with serum heavy chain in 7 (35%) patients. Use of FLC ratio instead of FLCs and "normalization" of urine FLCs based on concurrent urine and serum creatinine determinations did not change any of the correlations in any of the 23 participants (data not shown).

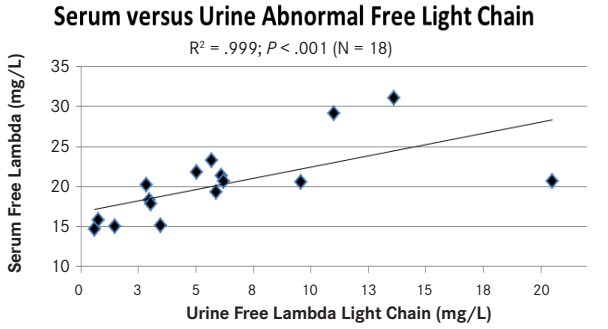
Figure 1 illustrates comparisons between urine and serum FLCs with serum heavy chains in 4 patients. Panels A and B illustrate patients for whom both urine and serum FLCs correlate with serum heavy chain; Panel C illustrates an individual for whom only the serum FLCs correlated with the serum heavy chain; and Panel D illustrates an individual for whom only the urine FLCs correlated with the serum heavy chain. **Figure 2** illustrates 2 patients for whom the urine FLCs correlated with the serum FLCs.

Discussion

Due to its greater sensitivity in the detection of monoclonal immunoglobulins, serum FLCs have become important for

FIGURE 2. Correlation in 2 Patients Between Abnormal/Monoclonal Serum and Urine FLCs.





FLC indicates free light chain; IgA, immunoglobulin A; and IgG, immunoglobulin G

diagnosis, prognosis, and management of plasma-cell disorders. This method is incorporated into current guidelines for assessing patients with monoclonal gammopathies. However, measurement of urine FLCs continues to be required because a small number of individuals are detected only by measurement of urine FLC excretion.[1,6]

Methodology for measuring urine FLCs continues to be collecting 24-hour urine specimen and extrapolating the quantity of light chains excreted based on urine IFE.[1,4] This process is subject to error when collection is incomplete, and the sensitivity of IFE is about 2-log less than the Freelite assay. Use of Freelite for measurement of urine FLCs has not been promoted because of a paucity of data using this method, and because of concern regarding using a spot urine due to variability in renal function and tubular handling of light chains,[10,13]

TABLE 2. Correlations Between Abnormal/Monoclonal Urine and Serum FLCs, and Between Abnormal/Monoclonal Urine and Serum FLCs and Abnormal/Monoclonal Serum Heavy Chain (P <.05)

Serum FLCs correlation to urine FLCs (N = 23)	9 (39%)
Urine and serum FLCs correlate with serum heavy chain (N = 20)	5 (25%)
Serum FLCs only correlate with serum heavy chain (N = 20)*	6 (30%)
Urine FLCs only correlate with serum heavy chain (N = 20)†	2 (10%)
No correlation between urine and serum FLCs and serum heavy chain (N = 20)	7 (35%)

* 4 individuals with active MM; 2 with MGUS.
† Both individuals with active MM.
FLC indicates free light chain; MGUS, monoclonal gammopathy of undetermined significance; and MM, multiple myeloma.

with prior studies failing to show association between urine FLCs and serum FLCs.[4,8,13] We also failed to show association with aggregated data. However, there was a clear association in 39% of patients between the serum and urine FLCs, and clear associations in 25% of patients between urine FLCs and serum heavy chain. We did not specifically compare head-to-head measurement of spot urine FLCs using Freelite with 24-hour collection quantitation via IFE; however, we feel that with the 2-log increased sensitivity of Freelite, Freelite will outperform IFE. We also observed a small but defined minority of patients for whom urine FLCs outperformed serum FLCs.

Use of Freelite as an assay for immunoglobulin light chains does not substitute for urinalysis to screen for albuminuria, which may be an indication of renal light-chain amyloid. If proteinuria is indicated on the screening urinalysis, a 24-hour urine collection should be considered to determine the quantity of albumin as a part of an evaluation for amyloid disease.

We feel that due to the increased (2-log) sensitivity of Freelite over IFE, the ability to quantitate the kappa-to-lambda light chain ratio as an indication of clonality, and the convenience of a spot urine over a 24-hour collection, this assay is a valid alternative to the currently promoted methodology.

Conclusion

The measurement of urine FLCs from a single urine collection using the Freelite assay is a convenient and valid tool, and in a minority of patients with monoclonal gammopathies, it adds unique information about disease activity.

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