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Comparison of 2 Serum-Free Light-Chain Assays in CKD Patients

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Introduction: Quantification of serum-free light chains (FLCs) is important in the diagnosis and monitoring of paraprotein-related diseases. There are currently 2 FLC assays available: the Freelite assay (Binding Site) and the N Latex assay (Siemens). There is emerging evidence that these assays give different results, but it is not established how kidney dysfunction affects these assays differently.

Methods: In this study, we measured and compared serum FLCs in patients with mild-to-moderate chronic kidney disease (CKD) using both assays.

Results: Although κ FLCs are higher by Freelite, λ FLCs are higher by N Latex. Both κ and λ FLCs correlate inversely with estimated glomerular filtration rate (eGFR) in the 2 assays, but this effect is more pronounced in λ-free light-chain measurement by N Latex. Consequently, although the κ/λ ratio by Freelite is inversely correlated by eGFR, the κ/λ ratio by N Latex is positively correlated with eGFR.

Conclusion: Our results clearly demonstrate that the 2 available FLC assays cannot be used interchangeably in patients with CKD.

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The quantification of FLCs in serum is nowadays an important tool in the detection and monitoring of clonal B-cell proliferative diseases. In the past decade, serum FLC measurement has become an important diagnostic tool in nephrology.[1] Freelite κ and λ reagents (The Binding Site, Birmingham, UK) were the first commercially available reagents to measure FLC in serum.[2] These reagents are based on polyclonal antibodies raised in sheep.[2] The clinical relevance of Freelite has been extensively demonstrated.[3–7] Since 2011, Siemens (Munich, Germany) has offered the N Latex test, which is based on monoclonal antibodies, for FLC measurement.[8] Monoclonal reagents have the advantage of high lot-to-lot consistency and reproducibility. A possible disadvantage is that epitopes can be missed.[9] It is increasingly appreciated that results obtained by the N Latex FLC assay may differ from results obtained by the Freelite assay, and these differences may have important implications.[8,10] To this date, direct comparison of serum FLC concentrations measured using the 2 available assays at the patient level in patients with mild-to-moderate CKD are scarce.[10–12] Jacobs *et al.*[10] have reported a comparison of the 2 available assays in 284 patients with varying degrees of CKD, including mild-to-moderate kidney dysfunction.

METHODS

Patient Samples

Serum samples from patients included in the Leuven mild-to-moderate kidney disease study were analyzed in this study. The Leuven mild-to-moderate kidney disease study is a prospective observational study in patients with prevalent CKD, followed at the CKD outpatient clinic of the University Hospitals Leuven. The study cohort has been reported and described in detail previously.[13] Of the original 499 patients included in this study, samples were available for 477 patients. Nine patients were excluded because of the

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presence of a B-cell hematologic malignancy at the time of presentation, resulting in 468 included in the current study. The study was performed according to the Declaration of Helsinki and approved by the ethics committee of the University Hospital Leuven. Informed consent was obtained from all patients. The trial was prospectively registered at clinicaltrials.gov (NCT00441623).

Baseline Biochemical Measurements

In the mild-to-moderate kidney disease study, demographics, smoking habit, presence of diabetes, prevalent cardiovascular disease, and cause of kidney disease were collected at time of informed consent. At inclusion, blood was taken by venous puncture for measurement of creatinine (mg/dl), hemoglobin (g/dl), biointact parathormone (ng/l), calcium (mg/dl), phosphate (mg/dl), albumin (g/dl), C-reactive protein (mg/l), cholesterol (mg/dl), and total and free p-cresol (mg/l). Creatinine, hemoglobin, parathormone, calcium, phosphate, C-reactive protein, and cholesterol were all measured using standard laboratory techniques. For the current analysis, the eGFR was calculated using the Chronic Kidney Disease–Epidemiology Collaboration equation.

FLC Measurement

Samples were stored frozen and thawed before analysis. All samples were run on the Optilite system using polyclonal Freelite reagents (The Binding Site) at the University Hospitals Leuven and on a BN ProSpec instrument using monoclonal N Latex reagents (Siemens) by the manufacturer according to their instructions.

Statistical Analysis

Method comparison was analyzed using Deming regression, under the assumption that there is no gold standard and that both methods have analytical bias. Deming regression provides estimates for both constant and proportional bias. Significance level was set at $P < 0.05$. Deming regression was performed using GraphPad (La Jolla, CA) Prism 8.

RESULTS

Comparison of κ and λ FLC Values Using the Freelite and the N Latex Assay

Overall, κ FLCs were higher by Freelite than by N Latex (Figure 1a). Values for λ FLC were in general higher by N Latex than by Freelite (Figure 1b). The κ/λ ratio was higher for Freelite than N Latex. This was not the case for values of κ/λ ratio <1 (by Freelite) (Figure 1c). The distribution of λ FLC values showed a discontinuous distribution for Freelite at a level of 7 mg/l, which is related to a switch in serum dilution (1:2 vs. 1:8), which results in a downward jump in λ FLC values below 7 mg/l using the Freelite assay.

Effect of eGFR on FLC Values Using the Freelite and N Latex Assay

Using the Freelite assay, it is well established that serum FLC concentration is influenced by renal function and adjusted normal ranges for FLC concentration and κ/λ ratio have been proposed for patients with CKD.[14] The effect of eGFR on FLC and κ/λ ratio using the N Latex assay is not well established at this time. We analyzed differences in values of κ FLC, λ FLC, and κ/λ ratio using the Freelite assay and N latex in patients stratified according to CKD stage and observed statistically significant differences in all CKD stages except for λ FLC in CKD stage Kidney Disease Improving Global Outcomes G1 and G2, and κ/λ ratio in CKD stage Kidney Disease Improving Global Outcomes G1 (Table 1). As the κ FLC is concerned, both in the Freelite assay and the N latex assay, there is an inverse relation between κ FLC and eGFR (Figure 1d). Also, for λ FLC, there is was an inverse relation between λ FLC and eGFR for both assays, but the effect of eGFR on λ FLC was more pronounced in the N Latex assay (Figure 1e). The effect of eGFR on the κ/λ ratio was opposite in the 2 assays: using the Freelite assay, the κ/λ ratio increases substantially with decreasing eGFR, whereas the effect of eGFR on the κ/λ ratio by N Latex is much less pronounced, but there is a decrease in κ/λ ratio with decreasing eGFR (Figure 1f). Although the association between decreasing eGFR and decreasing κ/λ ratio by N latex is statistically significant, the effect size is very small.

The Interassay Absolute Difference in κ and λ FLC and Effect of eGFR

The interassay absolute difference in κ FLC (κ FLC concentration by N Latex minus κ FLC concentration by Freelite) increases with decreasing eGFR and is negative as the κ FLC concentration by Freelite is greater than the κ FLC concentration by N Latex (Figure 1g). Also, the difference in absolute λ FLC values (λ FLC concentration by N Latex minus λ FLC concentration by Freelite) increased with decreasing eGFR (Figure 1h). The effect of eGFR on difference in λ FLC was more pronounced compared with the effect of eGFR on the difference in κ FLC, and the difference became positive as the λ FLC concentration by Freelite was smaller than the λ FLC concentration by N Latex (Figure 1h). Consequently, the interassay absolute difference in κ/λ ratio (κ/λ ratio by N Latex minus κ/λ ratio by Freelite) increases with decreasing eGFR and is negative as the κ/λ ratio is consistently higher by Freelite than by N Latex (Figure 1i).

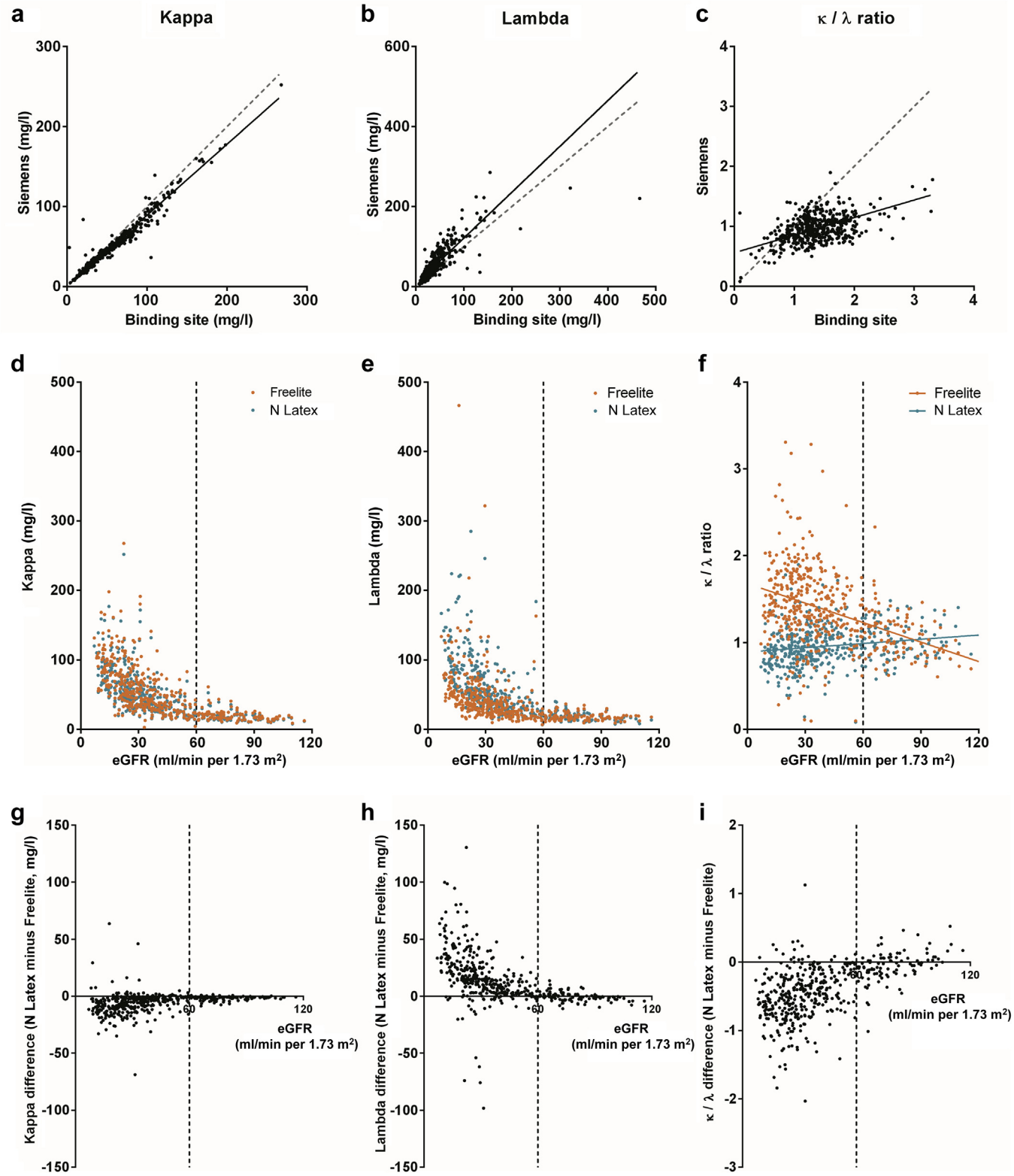

Figure 1. Deming regression analyses of (a) κ- and (b) λ-free light chains (FLCs), and (c) the κ/λ ratio, measured using the polyclonal antibody-based Freelite assay (Binding Site, Birmingham, UK) and the monoclonal antibody-based N Latex assay (Siemens, Munich, Germany). The absolute difference (N Latex minus Freelite) of the κ/λ ratio was plotted as a function of the estimated glomerular filtration rate (eGFR). Absolute concentrations (mg/l) of (d) serum κ FLCs and (e) serum λ FLCs as a function of the eGFR. The κ/λ ratio as a function of the eGFR (f). To illustrate the differential effect of the eGFR on the κ/λ ratio, linear regression curves are plotted. The concentration difference (N Latex minus Freelite) was plotted as a function of the eGFR for (g) κ light chains, (h) λ light chains. (i) Concentrations of individual patients measured using the Freelite are in orange. Samples measured using the Siemens assay are in blue.

Table 1. Mean and SD for the Freelite and N Latex assay per chronic kidney disease stage (according to KDIGO)

	Patients (*n*)	Freelite	N Latex	*P* value
Kappa				
KDIGO G1	31	15.8 (4.1)	14.6 (3.7)	<0.001
KDIGO G2	72	21.2 (8.6)	19.3 (7.3)	<0.001
KDIGO G3a	57	29.6 (15.3)	27.1 (12.4)	<0.001
KDIGO G3b	119	48.3 (29.5)	42.7 (24.3)	<0.001
KDIGO G4	142	71.3 (34.7)	63.3 (31.1)	<0.001
KDIGO G5	47	97.7 (31.2)	89.1 (30.0)	<0.001
Lambda				
KDIGO G1	31	17.4 (6.4)	15.1 (5.1)	0.0006
KDIGO G2	72	19.1 (6.1)	19.3 (6.5)	0.7
KDIGO G3a	57	25.9 (23.0)	30.9 (25.5)	<0.001
KDIGO G3b	119	35.0 (19.9)	44.1 (22.3)	<0.001
KDIGO G4	142	54.2 (51.2)	73.7 (43.0)	<0.001
KDIGO G5	47	68.4 (27.1)	103.7 (36.2)	<0.001
Kappa/lambda ratio				
KDIGO G1	31	0.95 (0.23)	1.01 (0.20)	0.11
KDIGO G2	72	1.12 (0.27)	1.01 (0.21)	0.0001
KDIGO G3a	57	1.28 (0.37)	0.99 (0.24)	<0.001
KDIGO G3b	119	1.42 (0.43)	0.98 (0.2)	<0.001
KDIGO G4	142	1.54 (0.51)	0.92 (0.25)	<0.001
KDIGO G5	47	1.50 (0.35)	0.88 (0.19)	<0.001

KDIGO, Kidney Disease Improving Global Outcomes.
Kappa- and lambda-free light-chain concentrations and ratios, stratified according to KDIGO stages 1–5 (not in dialysis). Data are reported as mean (SD), and differences were analyzed using paired *t* tests.

DISCUSSION

In this article, we compared the 2 currently available FLC assays: the Freelite assay (Binding Site) and the N Latex assay (Siemens) in patients with mild-to-moderate CKD. Our data allowed for the direct comparison of serum FLC concentrations obtained by the 2 currently available assays at the individual patient level.

As reported previously, κ FLCs are in general higher by Freelite than by N Latex.[15] In contrast, λ FLCs are higher by N Latex.[10,15] The κ/λ ratio is higher for Freelite than N Latex for values >1 (by Freelite), as we reported previously.[15] As FLC measurement is increasingly important in the diagnosis and monitoring of paraprotein-related kidney diseases, we were particularly interested to analyze the effect of kidney dysfunction on the 2 available FLC assays. In both assays, κ and λ FLC correlate inversely with eGFR, but this effect is more pronounced in λ FLC measurement by N Latex. Consequently, although the κ/λ ratio by Freelite is inversely correlated by eGFR, the κ/λ ratio by N Latex is positively correlated with eGFR. Although the association between decreasing eGFR and decreasing κ/λ ratio by N latex is statistically significant, the effect size is very small and clinically not relevant.

These are important observations, as they clearly demonstrate that the 2 available FLC assays cannot be used interchangeably in patients with kidney dysfunction. Currently, existing guidelines regarding FLC in B-cell clonal proliferative disorders are based on the Freelite assay. Physicians should be aware that the same reference intervals cannot be used for κ FLC, λ FLC, and κ/λ ratio by N Latex. Using the Freelite assay, there is a gradual increase of the κ/λ ratio with increasing degree of kidney dysfunction. So, for the Freelite assay, a renal reference interval must be applied when interpreting results, as has been reported.[1] In contrast, for the N latex assay, there is no need to use a renal reference interval, and the reported reference interval (0.31–1.56) can be applied for different degrees of kidney impairment.[8]

There are significant methodological differences between the 2 available FLC assays; although the Freelite κ and λ reagents are based on polyclonal antibodies, the N Latex test is based on monoclonal antibodies. How this results in different values for κ and λ FLC between the 2 assays is not clear. Even more puzzling is the different effect of eGFR on the different FLC assays, as there is currently no clear explanation for this discrepancy, although it has been suggested that it relates to a different reactivity to monomeric compared with dimeric forms of light chains.[16,17]

In conclusion, our results demonstrate that in patients with CKD, the 2 currently available FLC assays cannot be used interchangeably, as they are differently affected by renal dysfunction. For the N latex assays, no renal reference interval is needed, making it more straightforward to use. However, existing guidelines regarding FLC in B-cell clonal proliferative disorders are based on the Freelite assay and it is, based on our results, clear that defined cutoffs should be redefined using the N latex assay. Efforts should be undertaken to align and/or harmonize the clinical interpretation between the 2 assays.

DISCLOSURE

All the authors declared no competing interests.

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