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N Latex FLC serum free light chain assays in patients with renal impairment

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Introduction: The aim of this study was to establish ranges for N Latex free light chain (FLC) assays in patients with renal impairment.
Methods: Sera from 284 patients with chronic kidney disease (CKD) and 157 controls were measured with both N Latex and Freelite™ FLC reagents.
Results: Both κ-FLC and λ-FLC concentrations increased with the N Latex FLC and the Freelite™ assays with each increment in CKD stage. The median Freelite™ κ/λ--ratio in patients with severe renal failure was significantly increased compared to healthy controls and several samples were above the reference range for healthy controls (0.26 - 1.65). In contrast, none of the 284 patients with CKD had an FLC κ/λ--ratio exceeding the N Latex reference limits for healthy controls (0.31 - 1.56).
Conclusion: These findings demonstrate that the N Latex FLC κ/λ-ratio in patients with renal failure did not differ from the reference limits for healthy controls.

Key words: free light chains, renal failure, chronic kidney disease, N Latex FLC, Freelite

Serum free light chain (FLC) analysis plays a key role in diagnosing and monitoring patients with monoclonal gammopathies (1). The clearance of the relatively small FLC proteins occurs mainly through the kidney. Using Freelite™ assays to measure FLC, it was previously shown that a reduction in renal function causes an increase in circulating concentrations of κFLC and λFLC above the reference limits for normal healthy donors (2, 3). The κ/λ FLC-ratio is also significantly increased in patients with chronic kidney disease (CKD) and a modified κ/λ reference range of 0.37-3.1 for the Freelite™ FLC test was proposed to prevent that a significant number of patients with CKD are misclassified as having a κ monoclonal gammopathy (3).
In 2011, new monoclonal antibody-based assays for κFLC and λFLC became available for the BN systems of Siemens (4). The essential components of these N Latex FLC assays are mixes of monoclonal antibodies, in contrast to Freelite that use polyclonal antisera, to detect the available FLC-epitopes. Although both assays are not interchangeable with respect to the measurement of the absolute FLC concentrations, several studies have demonstrated the clinical value of these N Latex FLC assays (5, 6). The aim of this study was to determine the concentration of serum κFLC and λFLC with the N Latex FLC assays in patients with CKD and investigate whether special reference ranges are required for the calculated κ/λ-ratio when using the N Latex FLC assays in these patients.

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Materials and methods

Study population
For detailed information on the study population we refer to our previous publication (7). Briefly, for this retrospective study, patients with proven kidney damage, kidney failure or decreased kidney function for more than 3 months and creatinine ≥ 89 µmol/L or MDRD <60 mL/min/1.73 m^2 were included. Samples were no older than 1 year. Aliquots were stored at -20°C within three days after collection and thawed directly before analysis. All data analysis was coded and anonymized. Patients were classified according to KDOQI (Kidney Disease Outcomes Quality Initiative) into CKD1 to 5 as defined by the creatinine concentrations (7). Blood from CKD patients on dialysis was drawn pre-dialysis. Blood from157 patients without monoclonal gammopathy and renal impairment (creatinine < 89 µmol/L) from the Hoedemakers study served as controls (5).

Free light chain assays
The N Latex FLC assays were performed on the BN ProSpec® and BN™II (4). We performed the Freelite™ assays on a BN™II instrument with the special kits for BN™II. Both FLC assays were performed according to the manufacturers protocols. The reference ranges in healthy donors are as followed: N Latex κFLC 6.7–

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22.4 mg/L, Freelite™ κFLC 3.3–19.4 mg/L, N Latex λFLC 8.3–27 mg/L, Freelite™ λFLC 5.71–26.3 mg/L, N Latex FLC κ/λ-ratio 0.31–1.56, Freelite™ κ/λ-ratio 0.26–1.65 (2, 4). The modified Freelite™ κ/λ-ratio in patients with renal failure range from 0.37–3.1 (3).

Statistical analysis

Statistical analysis of the data was performed using the Microsoft Excel add-in Analyse-it® software (Analyse-it® v2.03, Method Evaluation, www.analyse-it.com) and GraphPad Prism® (v5.01, GraphPad Software Inc., www.graphpad.com).

Results

In patients with impairment renal function we measured significantly increased κFLC concentrations with each increment in CKD stage for both the N Latex FLC and the Freelite™ assays (fig. 1A and table 1). There was no difference between both methods for κFLC. Similarly, we found a significant increase in λFLC concentrations for both methods in patients with renal impairment (fig.1B and table 1). In the CKD5 group, the N Latex λFLC concentrations (median 128 mg/L) were significantly higher compared to the Freelite™ λFLC concentrations (median 89.5 mg/L, p<0.0001).

The FLC analyses in the dialysis group (57 patients) strongly resembled the results observed in the CKD5 group. For patients on dialysis, the N Latex κFLC concentrations were not significantly different from the Freelite™ κFLC concentrations. In contrast to this, the λFLC concentrations were significantly higher with the N Latex λFLC assay (median 147 mg/L) compared to the Freelite™ (median 89 mg/L, p<0.0001). These discrepancies between both assays mainly observed in λFLC, resulted in significant differences between Freelite™ κ/λ-ratios and N Latex FLC κ/λ-ratios in patients with CKD (fig.1C and table 1, P<0.02). The Freelite™ κ/λ-ratios were significantly increased in the CKD1, CKD5 and dialysis groups, compared to the Freelite™ control group (p<0.0001). In 11 out of the 66 patients in group CKD5 the Freelite™ κ/λ-ratio exceeded the reference limits for controls (0.26–1.65). In contrast, the N Latex FLC κ/λ-ratios in the CKD5 group and dialysis group were significantly lower than for the N Latex FLC control group (p<0.0001). In all patients with CKD, the N Latex FLC κ/λ-ratios were within the limits for healthy controls (0.31–1.56).

Discussion

In this study, we demonstrated that serum κFLC and λFLC concentrations measured both with the N Latex FLC and the Freelite™ assays are strongly correlated with CKD stage. Furthermore, we observed significantly different κ/λ-ratios in patients with CKD for the two tests. The Freelite™ κ/λ-ratio in patients with severe CKD was significantly increased compared to healthy controls and several individual samples were outside the reference range for healthy controls (0.26–1.65). In contrast, none of the 284 patients with CKD had an FLC κ/λ-ratio exceeding the N Latex reference limits for healthy controls (0.31–1.56). These results suggest that the two FLC assays perform structurally different in serum of patients with CKD. The current hypothesis, to explain the increased Freelite™ κ/λ-ratio seen in patients with renal insufficiency, states that as renal function declines, the reticuloendothelial clearance becomes increasingly important. As this route is presumably not influenced by the molecular weight of the FLC, the serum concentration in these patients reflects the FLC synthesis rate, which is higher for kappa (3). Since no international FLC standard or reference method is available, it is currently not possible to objectively determine which FLC method truly reflects the correct FLC concentrations in patients with renal insufficiency.

In conclusion, we show that the N Latex FLC detects

Table 1. Ranges for FLC in patient with CKD

	N Latex FLC						
		κFLC		λFLC		κ/λFLC	
	n	median	95% Range	median	95% Range	median	min - max
Control	157	17.2*	10.4 - 30.3	15,8	8.3 - 29.3	1.09$	0.68 - 1.57
CKD1	118	30.2#	14.9 - 66.4	32.5#	13.9 - 64.4	0.90$	0.49 -1.47
CKD2	33	41.2#	21.9 - 85.4	52.0#	21.6 - 95.2	1.00$	0.52 - 1.52
CKD3	34	53.5#	24.2 - 105	64.9#	24.7 - 139	0.85$	0.56 - 1.56
CKD4	25	65#	23.1 - 192	67#	30.2 - 254	0.82$	0.38 - 1.38
CKD5	74	85.8#	37.3 - 231	128* #	43.0 - 302	0.69## $	0.32 - 1.54
Dialysis	57	95.2#	34.7 - 186	147* #	42.3 - 309	0.62## $	0.35 - 1.48
	Freelite™						
		κFLC		λFLC		κ/λFLC	
	n	median	95% Range	median	95% Range	median	min - max
Control	157	15.5*	8.2 - 25.9	15,2	9.4 - 25.7	1.00$	0.29 - 2.37
CKD1	68	29.5#	16.9 - 66.4	29.9#	16.3 - 72.8	1.11# $	0.64 - 1.80
CKD2	19	42.0#	20.5 - 81.9	50.8#	21.4 - 94.6	0.88$	0.56 - 1.54
CKD3	21	54.4#	27.1 - 116	52.3#	25.8 - 116	1.05$	0.63 - 1.60
CKD4	16	76.1#	35.2 - 186	67.7#	45.1 - 189	0.95$	0.67 - 1.66
CKD5	66	98.1#	28.8 - 272	89.5* #	34.7 - 197	1.22# $	0.22 - 2.70
Dialysis	57	98.5#	28.3 - 256	89.0* #	34.4 - 186	1.23# $	0.22 - 2.36

# indicates a significant increase from the control group (Mann-Whitney U-test unpaired: p<0.0001)

indicates a significant decrease from the control group, significant difference between N Latex FLC and Freelite™ * p<0.0005 and $ p<0.02

increased concentrations for both κFLC and λFLC with each increment in CKD stage. The same N Latex FLC κ/λ-ratio reference interval can be used for both healthy controls and patients with renal failure.

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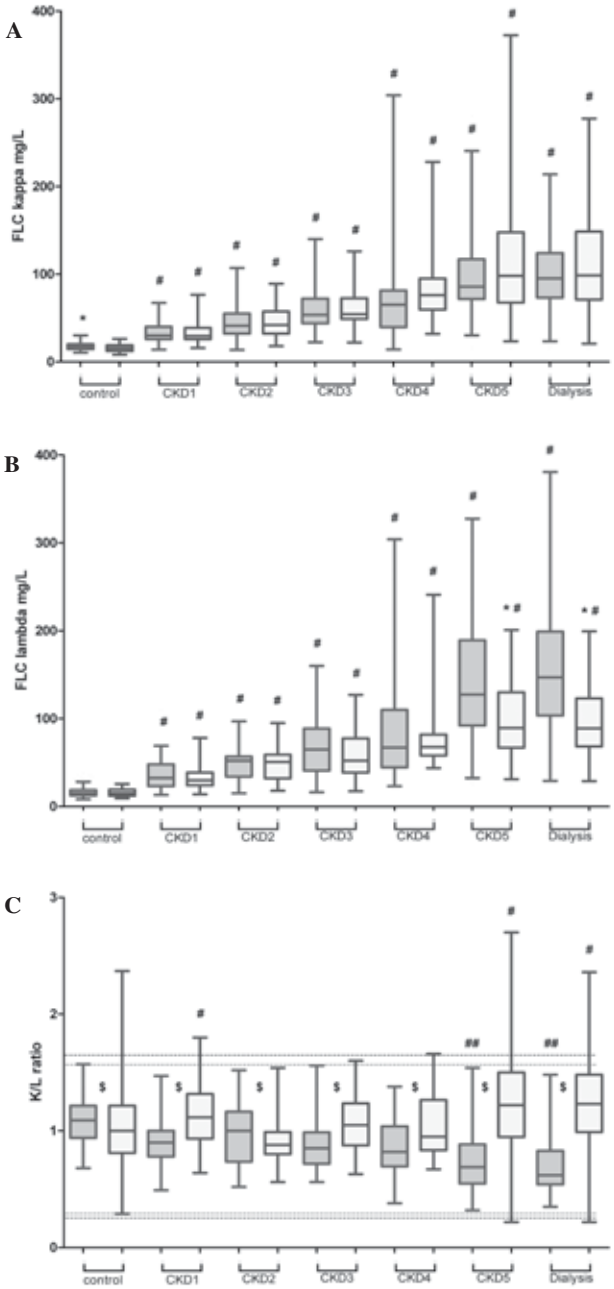

Figure 1. Box and Whiskers plots for κFLC (A), λFLC (B) and κ/λ-ratio (C) in patients with CKD stages 1-5.

Samenvatting

Jacobs JFM, Hoedemakers RMJ, te Velthuis H. N Latex FLC serum vrije lichte keten assays in patienten met een nierstoornis. Ned Tijdschr Klin Chem Labgeneesk. 2014;39:179-181
Introductie: doel van deze studie was het vaststellen van normaalwaarden voor N Latex vrije lichte ketens (VLK) in patiënten met een nierstoornis.
Methoden: Sera van 284 patiënten met chronische nierziekte en 157 controles werden gemeten met zowel N Latex en Freelite VLK assays.
Resultaten: De κ-VLK and λ-VLK concentraties, zowel gemeten met de N Latex en Freelite VLK assays, stegen bij toenemend verlies van nierfunctie. De mediane Freelite κ/λ--ratio in patiënten met ernstig nierfalen was significant hoger vergeleken met gezonde controles. Diverse sera hadden een κ/λ--ratio boven de normaalwaarde van 0,26-1,65. Dit was in tegenstelling tot de N Latex VLK assay, waarbij geen van de 284 patiënten met een nierstoornis een κ/λ--ratio boven de normaalwaarde van 0,31-1,56 had.
Conclusie: Onze bevindingen laten zien dat de N Latex VLK κ/λ-ratio in patiënten met een nierstoornis niet anders is dan de normaalwaarde voor gezonde controles.

Trefwoorden: vrije lichte ketens, nierfalen, chronische nierziekte, N Latex FLC, Freelite