Reference interval of free light chains ratio in patients with end-stage renal disease on chronic hemodialysis

The correct interpretation of serum free light chain (sFLC) results is vital for the diagnosis of any monoclonal gammopathy (MG). In patients with chronic kidney disease (CKD), the concentration of sFLC increases proportionally to the degree of reduction in glomerular filtration rate (GFR).[1] The currently used reference interval of the Kappa/Lambda sFLC ratio (FLCr) in patients with CKD is between 0.37 and 3.1;[2] however, studies on this matter have reported only a few patients on hemodialysis (HD).[3-5] Long *et al*. recently proposed new FLCr intervals based on CKD stage using estimated GFR (eGFR), but their study included only 8 patients on HD.[6] Considering the above, the normal reference interval of FLCr in patients on chronic HD remains unclear.

The aim of this study was to define the reference interval of sFLC and FLCr in patients with end-stage renal disease (ESRD) on chronic HD. The Local Ethics Committee approved the present work, and all the participants provided written informed consent.

Stable adult patients with ESRD on conventional thrice-weekly high-flux HD with a Helixone® plus membrane dialyzer (Fx Cordiax, Fresenius, Germany) for at least three months from a single dialysis center (Nephrocare Providencia, Santiago de Chile, Chile) were included. Demographics, comorbidities, and renal diagnosis data were taken from the dialysis center registry. All blood samples were collected on a single day, just before connection to the mid-week HD session. sFLC were measured using the Freelite® assay according to the supplier's instructions, using the Optilite 864 turbidimetric analyzer (The Binding Site, Birmingham, UK). In non-anuric patients, residual kidney function (RKF) was defined as 24-hour urine volume >250 mL, measured with a measuring jug in the interdialytic period. In patients with RKF, we calculated the eGFR using an equation based on plasma levels of β2-microglobulin.[7]

For MG detection, we performed capillary serum protein electrophoresis (SPEP) and sFLC to all patients. In patients with monoclonal spike, hypogammaglobulinemia or altered FLCr, immunosubtraction (Minicap Flex Piercing - Sebia) was also performed in order to get a better characterization of the paraprotein. Patients with incidental diagnosis of MG were excluded from the analysis, and were referred to the Hematology Unit for a more accurate diagnosis and management. Normality of data was assessed by the Kolmogorov-Smirnov test. Differences in continuous variables were compared with the use of a Student *t* test or Mann-Whitney U test. Double-sided 95% reference intervals were determined as recommended by the Clinical and Laboratory Standards Institute (CLSI Guidelines C28-A3),[8] using a non-parametric percentile method or a normal distribution method for data that exhibit parametric distribution. Atypical data (outliers) were evaluated with Tukey test, and not removed from the analysis. Correlations between sFLC and age and eGFR were assessed by Spearman rank-correlation coefficient. $P<0.05$ was considered statistically significant.

A total of 142 patients were initially included. An MG was incidentally detected in 5 patients (3.5%) who were excluded from the final analysis. Demographic and clinical characteristics of the 137 cases are shown in Table 1. A total of 27% of patients had RKF, with a median urine output in 24 hours of 700 mL (interquartile range [IQR], 448-1150) and a median eGFR of 3.42 mL/min (IQR, 1.36-9.74).

Kappa and Lambda values for the 137 patients are available in the *Online Supplementary Appendix*. The median Kappa level was 157 mg/L (IQR, 125.8-197.3), with a reference range of 64.5-281.8 mg/L. There was no significant relationship between Kappa levels and age of participants ($P=0.98$ by Spearman's correlation) (Figure 1A). The median of Kappa in patients with or without RKF was 126.6 mg/L (IQR, 98.2-152.9) and 165.6 mg/L (IQR, 132.4-202.4), respectively ($P<0.01$) (*Online Supplementary Appendix*). In patients with RKF, we found a moderate inverse correlation between eGFR and Kappa levels (Spearman's rank correlation coefficients, -0.51; $P<0.01$).

The median Lambda level was 139.4 mg/L (IQR, 112.1-184.6), and levels showed no significant correlation with age ($P=0.24$ by Spearman's correlation) (Figure 1B). The reference interval of Lambda was found to be between 47.1 and 312.6 mg/L. The median (IQR) in patients with RKF was 105.9 mg/L (IQR, 68.8-142) *versus* 147.8 mg/L (IQR, 120.5-189.9) in those without RKF ($P<0.01$) (*Online Supplementary Appendix*). Correlation analyses between Lambda levels and eGFR revealed significant negative correlation for patients with RKF (Spearman's rank correlation coefficients, -0.43; $P<0.01$).

Mean FLCr was 1.15 (Standard Deviation [SD], +0.31) and the reference interval was 0.55-1.75 (Table 2). Average FLCr was 1.23+0.32 SD and 1.12+0.3 SD in patients with and without RKF, respectively ($P=0.06$). Correlation analysis exhibited a weak but significant positive correlation between age and FLCr (Spearman's correlation coefficient, 0.18; $P=0.04$) (Figure 1C). No correlation was found between FLCr and eGFR ($P=0.48$). In patients with RKF, there was no correlation between residual urine output (mL/day) and sFLC levels or FLCr ($P=0.3$, 0.4, and 0.9 for Kappa, Lambda, and FLCr, respectively). Characteristics of patients with incidental MG diagnosis are shown in the *Online Supplementary Appendix*.

To date, this is the study with the largest number of patients that attempts to define the normal range of sFLC and the FLCr in this population. Our results demonstrate that, in

Table 1. Patients' baseline demographic and clinical characteristics.

Characteristic	Patients N=137
Age in years, mean ± SD	62.3±14.1
Age group in years, N (%)	
18-39	10 (7.3)
40-59	52 (38)
60-79	62 (45.2)
>80	13 (9.5)
Sex, N (%)	
Male	78 (56.9)
Female	59 (43.1)
Ethnicity, N (%)	
Latino/Hispanic	129 (94.2)
Other	8 (5.8)
Medical history, N (%)	
Diabetes mellitus	43 (31.4)
Hypertension	90 (65.7)
Heart failure	26 (19)
Kidney transplant	17 (12.4)
Cause of kidney disease, N (%)	
Diabetic kidney disease	34 (24.8)
Hypertensive or renovascular disease	24 (17.5)
Glomerular disease	19 (13.9)
Chronic interstitial nephritis or obstruction	14 (10.2)
Hereditary or cystic disease	11 (8)
Miscellaneous conditions	5 (3.6)
Uncertain or unrecorded cause	30 (21.9)
Median time on dialysis in mths (IQR)	78 (45.5-165.5)
Residual kidney function,* N (%)	37 (27)
Median body-mass index (IQR), Kg/m^2	26 (23.3-29.1)
Serum creatinine, mg/dL, mean ± SD	8.4±2.5
Hemoglobin, g/dL, mean ± SD	11.1±1.3
Median serum ferritin (IQR), ug/L	442 (221-595)
Serum albumin, g/dL, mean ± SD	4.±0.4
Calcium, mg/dL, mean ± SD	9.0±0.7
Median serum β-2 microglobulin, mg/L (IQR)	25.4 (16.2-32)
eKt/V, mean ± SD	1.53±0.3
Estimated dry weight, kg, mean ± SD	68.6±16.7

IQR: interquartile range; mths: months; SD: standard deviation. *Residual kidney function was defined as urine output >250 mL daily.

Table 2. Current recomendations of free light chains ranges, and the present results showing the suggested reference interval for hemodialysis patients.

eGFR mL/min/1.72/m^2	Kappa FLC mg/L	Lambda FLC mg/L	K/L FLC ratio
Normal[16]	3.3-19.4	5.7-26.3	0.26-1.65
eGFR 45-59[6]	7.8-83.6	7.3-65.1	0.46-2.62
eGFR 30-44[6]	8.8-103.3	8.2-73.2	1.48-3.38
eGFR <30[6]	11.7-265.1	12.6-150.9	0.54-3.30
ESRD in chronic HD*	64.5-281.8	47.1-312.6	0.55-1.75

eGFR: estimated glomerular filtration rate; ESRD: end stage renal disease; FLC: free light chain; HD: hemodialysis; K/L: Kappa/Lambda. *Data from the present study.

stable patients on chronic HD with high-flux filters, the reference interval for the FLCr is 0.55-1.75, closer to the range of patients without renal failure. Clearance with high-flux membranes of medium molecules such as sFLC has been optimized, which could explain our results.[9]

Previous studies exploring the reference interval of FLCr on patients with CKD have considered a heterogeneous population with CKD in different stages, but patients on HD have been under-represented. The first approaches were carried out by Hutchinson *et al*. considering 688 patients with CKD, of which only 22 patients had ESRD on HD.[2] The Iceland Screens, Treats or Prevents MM (iStopMM) study is a huge nationwide, prospective screening study where more than 75,000 participants were screened for M protein with SPEP, immunofixation, and sFLC. Recently, they have studied FLC reference ranges in patients with CKD (eGFR <60 mL/min/1.73m^2) at different stages. It included 6,503 patients, of whom only 8 patients were on chronic conventional HD, who were excluded from the main analysis.[6] The new interval suggested for all patients with CKD was 0.97-1.39. They grouped all patients with GFR <30 mL/min/1.73m^2 in only one category, where the suggested interval was 0.54-3.30. No specific reference intervals were presented for the subgroup of patients on HD.[6] Our results are complementary to this information.

Our Kappa and Lambda values were markedly higher than in the iSTOP study,[6] which did not stipulate nor describe the moment of sampling with respect to the HD session as did other studies and ours.[2,10] This highlights the need to establish the best timing for sampling. Considering that immediately before HD is the moment in which the highest concentrations of light chains are reached, these values should be more representative.

We incidentally diagnosed 5 cases of MG. The correct diagnosis of MG in patients on HD is complex, since they are patients who already have organ (renal) damage. Up to 11% of CKD patients may have MG, without necessarily having a causal correlation.[11] It is imperative to rule out multiple myeloma, because they require treatment to avoid other organ damage. It is worthy of note that, in cast nephropathy, involved FLC level is usually >500 mg/L or even higher according to the International Myeloma Working Group, so FLCr should be clearly altered.[12,13] AL amyloidosis or monoclonal immunoglobulin deposits disease are some diagnostic alternatives, although these pathologies tend to be systemic, and present more often with nephrotic syndrome than with ESRD. Another diagnostic possibility is an MG of renal significance (MGRS), which could have reached ESRD due to lack of early diagnosis and treatment. We diagnosed 3 patients with MGUS, but we must clarify that we were not able to properly differentiate an MGRS from an MGUS diagnosis due to the impossibility of having renal histology, without which the correct diagnosis cannot be made.[14]

On the other hand, our results imply an increase of light

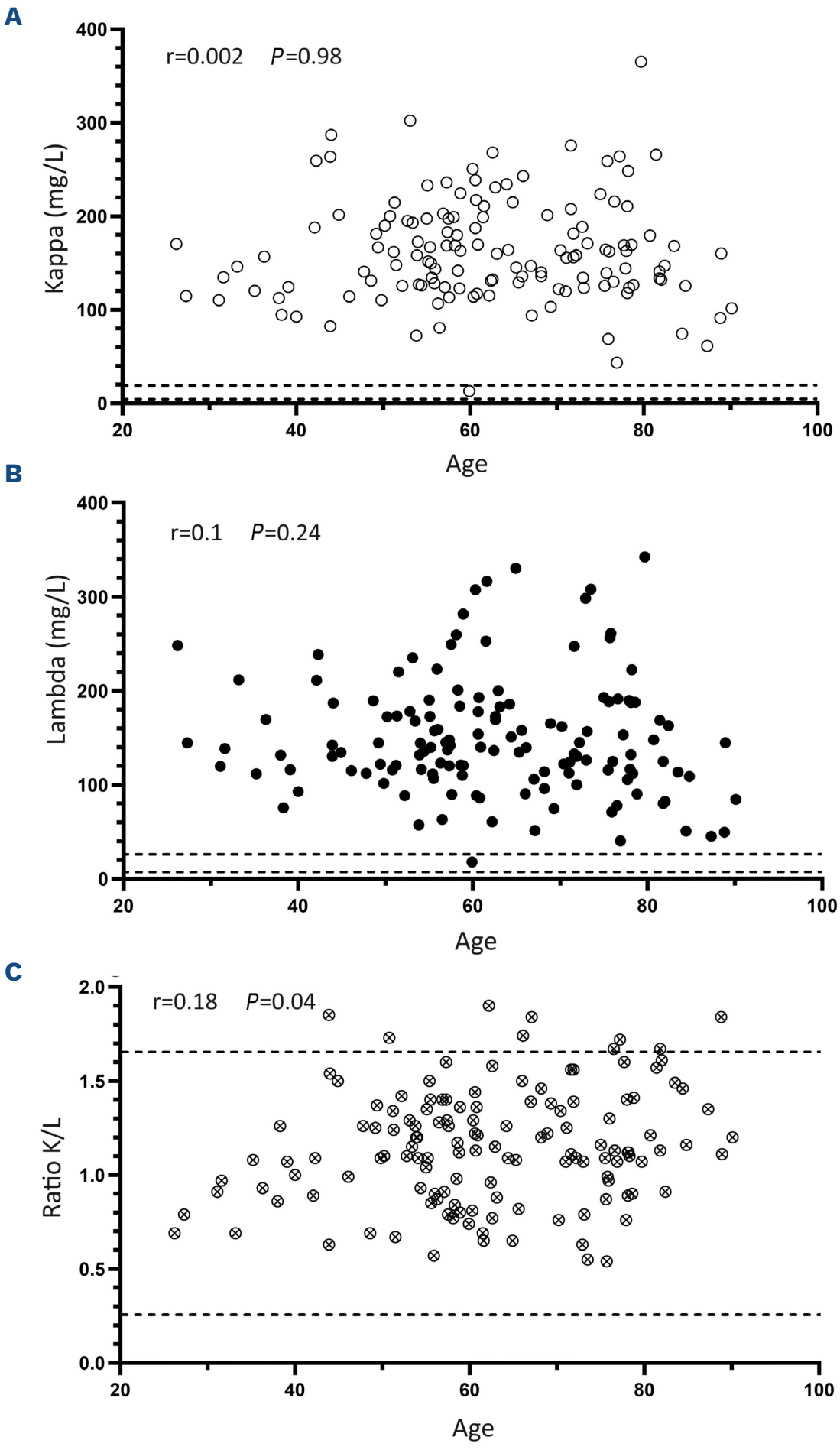

Figure 1. Correlation between serum free light chains and age of patients, and comparison with normal serum free light chain reference values. Dashed lines indicate the reference interval for patients without kidney disease. (A) Correlation between Kappa serum free light chain (sFLC) and age. (B) Correlation between Lambda sFLC and age. (C) Correlation between sFLC ratio with age of patients.

chain (LC)-MGUS diagnosis in patients on HD. These results should be taken with caution since it can lead to extra psychological stress for patients. As in LC-MGUS in patients without HD, we should interpret the results judiciously, and consider FLCr >8 as patients at higher risk of progression.[15] Some limitations of this study are that it was performed at a single center and a single High-Flux dialyzer was used, so the obtained results cannot be completely extrapolated to other membranes or techniques. Moreover, there are other FLC assays in use, other than Freelite®, that give different results.

In conclusion, we propose new reference intervals for sFLC and FLCr in patients on stable chronic HD. We believe that our results allow better identification of people with true MG on chronic HD in the current era, the main concept being that the reference interval in patients on HD with high-flux membranes is closer to that of patients without renal failure.

Authors

Camila Peña,[1,2*] Ricardo Valjalo,[3*] Ramón Pérez,[4] Marco Álvarez,[5] Pablo Bustamante,[5] Esteban Forray,[5] Viviana Balboa[4] and Alexis Bondi[4]

[1]Hematology Section, Hospital del Salvador; [2]Centro de Investigación Clínica Avanzada (CICA-Oriente); [3]Nephrology Section, Hospital del Salvador; [4]Immunology Laboratory, Hospital del Salvador and [5]Internal Medicine Section, Hospital del Salvador, Santiago de Chile, Chile

**CP and RV contributed equally as first authors.*

Correspondence:
C PEÑA - cpena@hsalvador.cl

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Contributions

CP and RV designed the study. MA, PB and EF controlled the database. CP, RV, MA, PB and EF contributed to obtaining the clinical data. RP, VB and AB contributed to obtaining laboratory results. CP and RV performed the statistical analysis. All authors interpreted the data and wrote the manuscript, and read and approved the final version of the manuscript.

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Data-sharing statement

Data that support the findings of this study are available from the corresponding author upon request.

References

1. Miettinen TA, Kekki M. Effect of impaired hepatic and renal function on bence jones protein catabolism in human subjects. Clin Chim Acta. 1967;18(3):395-407.
2. 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.
3. Sprangers B, Claes K, Evenepoel P, et al. Comparison of 2 serum-free light-chain assays in CKD patients. Kidney Int Rep. 2020;5(5):627-631.
4. Desjardins L, Liabeuf S, Lenglet A, et al. Association between free light chain levels, and disease progression and mortality in chronic kidney disease. Toxins (Basel). 2013;5(11):2058-2073.
5. Jacobs JF, Hoedemakers RM, Teunissen E, Te Velthuis H. N Latex FLC serum free light-chain assays in patients with renal impairment. Clin Chem Lab Med. 2014;52(6):853-859.
6. 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.
7. Shafi T, Michels WM, Levey AS, et al. Estimating residual kidney function in dialysis patients without urine collection. Kidney Int. 2016;89(5):1099-1110.
8. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline-3rd ed. CLSI document EP28-A3c. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2008.
9. Vanholder R, De Smet R, Glorieux G, et al. Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int. 2003;63(5):1934-1943.
10. Kennard A, Hawley C, Tate J, et al. Comparison of freelite and N latex serum free light chain assays in subjects with end stage kidney disease on haemodialysis. Clin Chem Lab Med. 2016;54(6):1045-1052.
11. Fenton A, Chinnadurai R, Gullapudi L, et al. Association between non-malignant monoclonal gammopathy and adverse outcomes in chronic kidney disease: a cohort study. PLoS Med. 2020;17(2):e1003050.
12. Yadav P, Sathick IJ, Leung N, et al. Serum free light chain level at diagnosis in myeloma cast nephropathy-a multicentre study. Blood Cancer J. 2020;10(3):28.
13. Leung N, Rajkumar SV. Multiple myeloma with acute light chain cast nephropathy. Blood Cancer J. 2023;13(1):46.
14. Leung N, Bridoux F, Batuman V, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. Nat Rev Nephrol. 2019;15(1):45-59.
15. Gonsalves WI, Rajkumar SV. Monoclonal gammopathy of undetermined significance. Ann Intern Med. 2022;175(12):ITC177-ITC192.
16. Katzmann JA, 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;48:1437-44.