



Letter to the Editor

Biological variation of free light chains in serum

To the Editor,

In 2009, the International Myeloma Working Group recommended the measurement of serum immunoglobulin-free κ and λ light chains (FLC) and κ/λ FLC ratio calculation for screening, prognostic evaluation, and monitoring of multiple myeloma and related plasma cell disorders (PCD) [1]. In spite of a well defined clinical role, no reliable data on biological variation (BV) of FLC have been published yet. The only two studies available in literature evaluating FLC BV suffered indeed of important limitations, such as, e.g., analyses carried out in singlicate on only two individual samples collected one week apart [2] or evaluation of variability of monoclonal FLC in patients affected by PCD [3], a situation that markedly differ from the condition required by the theory of BV, which aims to define the physiological fluctuation of analyte concentrations in a biological fluid around its homeostatic set point [4]. Here we report a definitive assessment of analytical and biological components of the variation of κ and λ FLC and FLC ratio by an accurately designed experimental and statistical protocol.

We collected five blood specimens from each of 21 healthy volunteers (9 men and 12 women; age range, 23–54 years) on the same day, every two weeks for two months. Ostensibly healthy subjects were studied to ensure that any FLC fluctuation in serum could truly reflect biology and not modifications due to pathological processes. In accordance with the Helsinki II Declaration, the design and execution of the experiment were explained thoroughly to the subjects, and informed consent was obtained. The inclusion criteria were that the subjects have no evident hematological disorders, and none of the subjects took any medication or consumed substantial (> 10 g of ethanol/day) quantities of alcohol. Venous blood was obtained at 0900 from the subjects who had fasted for 12 h and had not smoked or exercised in that morning. Samples were collected by the same phlebotomist with minimal stasis using vacuum collection tubes with no anticoagulants or gel for serum separation. Serum specimens, separated by centrifugation, were stored at -80 °C until analysis. When all of the specimens were available, they were thawed, mixed, centrifuged, and analyzed in a single run in duplicate in random order. FLC were determined using a SPA_{PLUS} immunoturbidimetric platform and Freelite reagents both from The Binding Site. Cochran's test was performed for outlier identification among observations and within-subject variances, whereas Reed's criterion was used for identification of outliers among mean values of subjects [4]. After outlier exclusion, Shapiro–Wilk test was applied separately to the set of results from each individual to check data distribution. The analytical, within-subject, and between-subjects components of variation were calculated by nested ANOVA from replicate analyses [4]. Analytical variance was estimated from the duplicate results for each specimen, within-subject biological variance from the within-subject total variance (referred to as the variance of the mean of duplicate assays) minus one-half of the analytical variance, and between-subjects biological variance from the total variance of the data minus the analytical and intra-individual components. All the components of

variance were then transformed to the relevant CV using the overall means. The index of individuality, yielding information about the utility of conventional population-based reference intervals; the reference change value (RCV), i.e., the minimal significant difference ($P < 0.05$) between two consecutive FLC measurements in the same subject; and the number of specimens that should be collected to estimate ($P < 0.05$) the homeostatic set point of an individual within $\pm 10\%$ were estimated [4]. Finally, optimal, desirable, and minimum analytical goals for imprecision, bias, and total error for FLC and FLC ratio were obtained from BV components [5].

Table 1 shows the obtained results after removing the outliers. For all parameters Shapiro–Wilk test accepted the normality hypothesis in a substantial proportion ($\geq 94\%$) of subjects. Serum λ FLC concentrations were significantly ($P < 0.01$) higher for men than those for women, whereas no difference was found for κ FLC and FLC ratio. Intra-individual variances were not different between genders, while the inter-individual variance of λ FLC was higher than that of κ FLC. All parameters had a low index of individuality showing that classical reference intervals have little use in the interpretation of FLC results. For PCD monitoring, the knowledge of RCV is of pivotal importance for the critical evaluation of the significance of changes in the results obtained from analysis of serial samples in the same individual. From our data, an average RCV of $\sim 20\%$ can be assumed as a figure to guide clinical decision making, even if this information cannot be used by other laboratories working with different analytical imprecision. This RCV finding compares lower to a previously published RCV (54.5%) derived from the estimate of monoclonal FLC variability in patients with PCD [3]. It has previously been demonstrated that the presence of disease may significantly amplify the fluctuation of analyte concentrations around its set point and this can therefore explain this difference [6]. According to this ground, the smaller variability of FLC ratio, when compared with the data in the referenced disease cohort, is also better fitting with the reason of its clinical use directed to minimize FLC variations due to hydration status and hematocrit value [7].

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References

- [1] Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia* 2009;23:215–24.
- [2] Tate JR, Gill D, Cobcroft R, Hickman PE. Practical considerations for the measurement of free light chains in serum. *Clin Chem* 2003;49:1252–7.
- [3] Katzmann JA, Snyder MR, Rajkumar SV, Kyle RA, Therneau TM, Benson JT, et al. Long-term biological variation of serum protein electrophoresis M-spike, urine M-spike, and monoclonal serum free light chain quantifications: implications for monitoring monoclonal gammopathies. *Clin Chem* 2011;57:1687–92.
- [4] Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 1989;27:409–37.
- [5] Fraser CG, Hyltoft Petersen P, Libeer JC, Ricos C. Proposal for setting generally applicable quality goals solely based on biology. *Ann Clin Biochem* 1997;34:8–12.

Table 1Mean values, estimated average analytical (CV_A), intra-individual (CV_I) and inter-individual (CV_G) variations, and derived indices for serum free light chains (FLC) and κ/λ FLC ratio.

Parameter	Group (n)	Mean ^a	CV_A , %	CV_I , %	CV_G , %	II	RCV, %	n	Analytical goals								
									Imprecision as CV, %			Bias, %			Total error, %		
									Minimal	Desirable	Optimal	Minimal	Desirable	Optimal	Minimal	Desirable	Optimal
κ FLC	All (18)	7.96	1.2	8.1	14.1	0.33	22.6	2	≤ 6.0	≤ 4.0	≤ 2.0	$\leq \pm 6.1$	$\leq \pm 4.1$	$\leq \pm 2.0$	$\leq \pm 16.1$	$\leq \pm 10.7$	$\leq \pm 5.4$
	Men (7)	7.96		9.8	13.2	0.55											
	Women (11)	7.96		6.7	15.3	0.20											
λ FLC	All (19)	8.51	0.9	7.0	27.5	0.07	19.6	2	≤ 5.3	≤ 3.5	≤ 1.7	$\leq \pm 10.6$	$\leq \pm 7.1$	$\leq \pm 3.5$	$\leq \pm 19.3$	$\leq \pm 12.9$	$\leq \pm 6.4$
	Men (8)	8.97		6.6	33.0	0.04											
	Women (11)	8.18		7.3	22.6	0.11											
FLC ratio	All (17)	0.98	1.7	4.5	15.3	0.09	13.4	1	≤ 3.4	≤ 2.3	≤ 1.1	$\leq \pm 6.0$	$\leq \pm 4.0$	$\leq \pm 2.0$	$\leq \pm 11.6$	$\leq \pm 7.7$	$\leq \pm 3.9$
	Men (8)	1.00		4.2	12.7	0.12											
	Women (9)	0.97		4.8	18.0	0.07											

II, index of individuality; RCV, reference change value; n, number of blood specimens that should be collected to estimate the homeostatic set point of an individual.

^a Results of κ and λ FLC are in mg/L.

[6] Braga F, Dolci A, Mosca A, Panteghini M. Biological variability of glycosylated hemoglobin. Clin Chim Acta 2010;411:1606-10.

[7] Bradwell A, Harding S, Fourrier N, Mathiot C, Attal M, Moreau P, et al. Prognostic utility of intact immunoglobulin Igk/Igλ ratios in multiple myeloma patients. Leukemia 2012 Jun 13 doi:10.1038/leu.2012.159 [Epub ahead of print].

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