



## Letter to the Editor

**Biologic variation of copper, ceruloplasmin and copper/ceruloplasmin ratio (Cu:Cp) in serum**

To the Editor,

Diagnostic algorithms for Wilson's disease (WD) recommend the determination of serum ceruloplasmin in addition to the slit-lamp examination required to identify Kayser–Fleischer rings as clues in order to decide on or decline further testing, while measurements of total serum copper and/or ceruloplasmin-unbound (“free”) copper are of value in monitoring pharmacotherapy [1,2]. The direct measurement of “free” copper is, however, difficult and not routinely available [3]. To overcome this issue, equations have been proposed for assessing “free” copper fraction in serum; however, they can produce biologically implausible negative results in a significant number of patients [4]. The use of copper/ceruloplasmin ratio (Cu:Cp) has been proposed to overcome such problems [5].

Despite their clinical role, aspects related to biologic variation (BV) of total copper and ceruloplasmin in serum have not received enough attention, while information on “free” copper BV is totally lacking. We could retrieve only one published study carefully determining the BV of total copper using a well designed protocol [6]. Here we performed an assessment of BV components of total copper and ceruloplasmin concentrations in serum, and derived Cu:Cp, in the same cohort of subjects by an accurately designed protocol. We collected five blood specimens from each of 19 healthy volunteers (10 men and 9 women; age range, 23–48 years) on the same day, every two weeks for two months. Ostensibly healthy subjects were studied to ensure that any copper and ceruloplasmin fluctuation in serum could truly reflect biology and not modifications due to pathologic (e.g., inflammatory) processes. In accordance with the Helsinki II Declaration, the study design was explained thoroughly to the subjects, and informed consent was obtained. None of subjects took any medication or consumed substantial (> 10 g/day) quantities of alcohol. Women had regular menstrual cycle and did not use hormonal contraceptives. Venous blood was obtained at 0900 from 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. After centrifugation, serum specimens were stored at  $-80^{\circ}\text{C}$  until analysis. When all specimens were available, they were thawed and analyzed in a single run in duplicate in random order. Copper and ceruloplasmin were determined on Roche Cobas c501 analyzer using a colorimetric and an immunoturbidimetric assay, respectively. Cu:Cp was calculated by the

formula:  $\text{copper } (\mu\text{mol/L}) \times 0.132 / \text{ceruloplasmin } (\text{g/L})$  [5]. Cochran's test was performed for outlier identification among observations and among intra-individual variances, whereas Reed's criterion was used for identification of outliers among mean values of subjects [7]. The analytical, intra-individual and inter-individual components of variation were calculated as previously reported [8]. All the components of variance were then transformed to the relevant coefficient of variation (CV) using the overall means. The index of individuality (II), yielding information about the utility of conventional population-based reference intervals, the number of specimens ( $n$ ) that should be collected to estimate ( $P < 0.05$ ) the homeostatic set point of an individual and the reference change value (RCV) were estimated for each analyte [7]. Finally, optimal, desirable and minimum analytic goals for imprecision, bias and total error for analyte measurements were derived from BV components [9].

Table 1 shows the results after removing outliers. Cu:Cp was slightly higher ( $P = 0.02$ ) in women than in men, whereas no sex-dependent difference was found for copper and ceruloplasmin concentrations. Intra-individual variances of studied parameters were not different between genders. On the other hand, women showed ~2.5 times higher inter-individual variability ( $CV_C$ ) for copper and ceruloplasmin than men, a difference that disappeared for Cu:Cp. Cu:Cp also showed the smallest BV with a higher II. Therefore, if classical reference intervals have little use in the interpretation of serum copper and ceruloplasmin results, they are useful for interpretation of Cu:Cp values, mainly in women [7].

Although the diagnosis of WD necessitates the determination of ceruloplasmin in serum, clinical guidelines do not specify the number of measurements required to correctly estimate the individual set point for this analyte [1,2]. Based on our results it is now possible to integrate recommendations by introducing the need of two measurements for the correct estimation of individual ceruloplasmin concentrations into clinical practice. In the monitoring of disorders of copper metabolism the knowledge of RCV is of pivotal importance for the critical evaluation of the significance of changes in results obtained from analysis of serial samples in the same individual. From our data, an average RCV of 19% for both copper and ceruloplasmin can be assumed as a figure to guide clinical decision making, even if this information cannot be directly used by other laboratories working with different analytical variability. The smaller variability of Cu:Cp, when compared with those of copper and ceruloplasmin, is fitting with the reason of its clinical use directed to minimize variations in each of the two analytes [5]. However, Cu:Cp has the potential weakness to behave differently depending on the ceruloplasmin assay, as the measurement of this protein is not standardized [10].

**Acknowledgments**

We thank Fatima Calderaro for skilful technical assistance and Roberta Mozzi for drawing blood samples. We also acknowledge all

*Abbreviation:* WD, Wilson's disease; Cu:Cp, copper/ceruloplasmin ratio; BV, biologic variation; CV, coefficient of variation; II, the index of individuality;  $n$ , number of specimens that should be collected to estimate the homeostatic set point of an individual; RCV, reference change value;  $CV_C$ , inter-individual variability.

**Table 1**  
Mean values, estimated average analytic ( $CV_A$ ), intra-individual ( $CV_I$ ), and inter-individual ( $CV_G$ ) variability, and derived indices for serum copper, ceruloplasmin and copper/ceruloplasmin ratio (Cu:Cp).

Parameter	Group (n)	Mean	$CV_A$ , %	$CV_I$ , %	$CV_G$ , %	II	n	RCV, %	Analytic goals									
									Imprecision as CV, %			Bias, %			Total error, %			
									Minimal	Desirable	Optimal	Minimal	Desirable	Optimal	Minimal	Desirable	Optimal	
Copper, $\mu\text{mol/L}$	All (19)	11.5	3.7	5.8	14.5	0.19	2	18.9	$\leq 4.3$	$\leq 2.9$	$\leq 1.4$	$\leq \pm 5.9$	$\leq \pm 3.9$	$\leq \pm 2.0$	$\leq \pm 13.0$	$\leq \pm 8.7$	$\leq \pm 4.3$	
	Men (10)	11.4		6.6	7.5	0.90												
	Women (9)	11.7		4.8	19.7	0.07												
Ceruloplasmin, g/L	All (18)	0.20	2.8	6.2	14.4	0.21	2	19.0	$\leq 4.7$	$\leq 3.1$	$\leq 1.6$	$\leq \pm 5.9$	$\leq \pm 3.9$	$\leq \pm 2.0$	$\leq \pm 13.6$	$\leq \pm 9.1$	$\leq \pm 4.5$	
	Men (9)	0.20		7.3	8.4	0.82												
	Women (9)	0.20		4.9	19.4	0.07												
Cu:Cp, $\mu\text{mol/g}$	All (18)	7.7	4.6	2.4	3.8	1.15	1	14.3	$\leq 1.8$	$\leq 1.2$	$\leq 0.6$	$\leq \pm 1.7$	$\leq \pm 1.1$	$\leq \pm 0.6$	$\leq \pm 4.7$	$\leq \pm 3.1$	$\leq \pm 1.6$	
	Men (9)	7.5		1.3	3.5	1.06												
	Women (9)	7.9		3.2	2.2	4.01												

II, index of individuality; n, number of specimens that should be collected to estimate the homeostatic set point of an individual within  $\pm 10\%$ ; RCV, reference change value.

those who provided blood specimens for their willing and enthusiastic participation.

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9 November 2012