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Copper:caeruloplasmin ratio

Patrick J Twomey, Adie Viljoen, Ivan M House, Timothy M Reynolds, Anthony S Wierzbicki

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Investigation of copper status can be a diagnostic challenge. The non-caeruloplasmin-bound copper (NCC) has deficiencies; accordingly, the copper:caeruloplasmin ratio has been suggested as an alternative index of copper status. A reference interval for this index was derived. In addition to making the interpretation of copper easier, the copper:caeruloplasmin ratio should also enable adjustment for relatively high caeruloplasmin concentrations without recourse to producing gender- and age-derived intervals. The copper:caeruloplasmin ratio has weaknesses similar to those identified for NCC in that immunological methods used for caeruloplasmin can cross react with apocaeuloplasmin and there is no standardised method for caeruloplasmin. Caeruloplasmin assays also have uncertainty from precision, bias and specificity and, accordingly, method-related differences may have a large effect on the copper:caeruloplasmin ratio in a manner similar to the NCC.

Copper studies are performed to identify copper deficiency or excess. Wilson's disease (primary copper excess), an autosomal recessive condition,¹ results in copper deposition in the hepatic parenchymal cells, brain, iris and kidney. Its diagnosis can be a challenge.² Secondary copper excess is rare in the developed world. Childhood primary copper deficiency has a poor prognosis and is often caused by an inherited defect in copper absorption (Menke's disease).³ Adult primary copper deficiency can cause a neurological condition similar to the extrapyramidal signs of Wilson's disease.⁴ Secondary copper deficiency is possible in patients receiving long-term enteral nutrition⁵ and with overuse of zinc supplementation.⁶ Copper studies are often requested to diagnose copper deficiency and to prevent neurological deterioration with supplementation.

The vast majority of copper in serum is bound to caeruloplasmin.⁷ Laboratories often do not take age and gender into account when reporting serum copper and caeruloplasmin reference intervals.⁸ Furthermore, caeruloplasmin has 6–8 copper atoms per molecule, with the majority being tightly bound.^{9–10} Heterogeneity in the number of copper atoms per caeruloplasmin molecule is possible, so any formula assuming

six copper atoms per molecule may be valid only in certain situations.

Caeruloplasmin largely determines the copper concentration,^{11–12} and plays a critical role in the interpretation of copper results. Some patients with Wilson's disease have copper and caeruloplasmin within their respective reference intervals,^{1–9} usually with copper being in excess of the caeruloplasmin concentration. Furthermore, about 2% of the population are heterozygous for P-type ATPase mutations,¹ and often have caeruloplasmin results around the lower reference interval. Non-caeruloplasmin-bound copper (NCC) has been advocated as a superior diagnostic tool for Wilson's disease¹¹ to try to overcome such issues. There are problems associated with the current use of the NCC; in particular, it produces physiologically impossible negative results in a significant number of patients.¹² To be effective in distinguishing patients with Wilson's disease from normal patients, the NCC may be expressed as a percentage of total copper. Furthermore, any index for the study of copper status should ideally have the potential to be used in the investigation of excess and deficiency copper states. Accordingly, new indices have been proposed, such as copper corrected for caeruloplasmin¹³ and the copper:caeruloplasmin ratio.¹⁴ The first study included only normal patients, whereas the second included patients with Wilson's disease who predominantly presented with neurological symptoms. We derived our own reference interval for this index.

Using the copper and caeruloplasmin data for 338 patients without Wilson's disease or copper deficiency,¹² we calculated the copper:caeruloplasmin ratio ($[\text{copper}] \times 0.132 / [\text{caeruloplasmin}]$) where copper is in $\mu\text{mol/l}$ and caeruloplasmin is in g/L .

Mean, median, SD and 2.5th and 97.5th centiles by both parametric and non-parametric methods for the copper:caeruloplasmin ratio were derived from measurements of copper (Varian Spectra 20) and caeruloplasmin (Tinaquant Kit; Hitachi 912; Roche, Welwyn, Hertfordshire, UK) assays. The copper:caeruloplasmin ratio ranged from 3.9 to 12.97 $\mu\text{mol/g}$, with the mean and median being 6.81 and 6.83 $\mu\text{mol/g}$, respectively. The SD was 0.87 $\mu\text{mol/g}$ and, accordingly, the parametric reference interval was 5.08–8.55 $\mu\text{mol/g}$ and the non-parametric 2.5th

Abbreviation: NCC, non-caeruloplasmin-bound copper

Take-home messages

- Investigation of copper status can be a diagnostic challenge.
- The non-caeruloplasmin-bound copper has deficiencies.
- The copper:caeruloplasmin ratio should produce values that are independent of gender and age.
- The copper:caeruloplasmin ratio, unlike non-caeruloplasmin-bound copper, does not suffer from negative values; however, the other deficiencies of non-caeruloplasmin-bound copper also apply to the copper: caeruloplasmin ratio.

and 97.5th reference intervals using MS Excel were 5.05–8.09 $\mu\text{mol/g}$. Analysing copper by Varian SpectraAA220G and caeruloplasmin by the Beckman–Coulter Array (High Wycombe, Buckinghamshire, UK), Beetham¹⁴ derived a ratio range of 4.0–8.5 $\mu\text{mol/g}$. The difference at the upper end between the ranges may be due to either different analytical methods or the greater numbers in our study (338 vs 47 patient samples). After removing four outliers, Beetham's range was 5.2–7.3 $\mu\text{mol/g}$,¹⁴ which is closer to our parametric and non-parametric reference intervals (5.08–8.55 and 5.05–8.09 $\mu\text{mol/g}$, respectively). As our reference intervals were 10–25 $\mu\text{mol/l}$ and 200–500 mg/l for copper and caeruloplasmin, respectively, the mean copper:caeruloplasmin ratio should theoretically be 6.6 $\mu\text{mol/g}$, which is similar to both the study mean of 6.81 $\mu\text{mol/g}$ and the theoretical mean of 6.7 $\mu\text{mol/g}$ in Beetham's study. As stated by Beetham, this serves as a useful internal quality control check, providing an independent verification of caeruloplasmin calibration.

Beetham investigated seven patients with confirmed Wilson's disease and found a copper:caeruloplasmin ratio range of 9.0–44.0 $\mu\text{mol/g}$; all these results would be above the upper reference limit using our methods. However, 0.3% (4/338) values exceeded 9.0 $\mu\text{mol/g}$ in our population. Such patients may be normal or may be heterozygous for P-type ATPase mutations.¹ The positive and negative predictive values of the copper:caeruloplasmin ratio would be acceptable at 63.6% and 100%, respectively. Use of the parametric upper reference limit produces the same predictive values as above, whereas the non-parametric reference interval would have a positive predictive value of 46.7% while retaining a 100% negative predictive value, which is of the utmost importance in screening.

The copper:caeruloplasmin ratio is not just a way of making the interpretation of copper easier. It should enable adjustment for relatively high caeruloplasmin concentrations without recourse to producing gender- and age-derived intervals.^{8,9} The copper:caeruloplasmin ratio has weaknesses similar to those identified for NCC¹² in that immunological methods used for caeruloplasmin can cross react with apocaeuloplasmin and there is no standardised method for caeruloplasmin.⁹ Caeruloplasmin assays also have uncertainty from precision, bias and specificity¹⁵ and, accordingly, method-related differences may have a large effect on the copper:caeruloplasmin ratio in a manner similar to the NCC.

The copper:caeruloplasmin ratio does not have negative values for samples and overcomes the need for gender and age reference interval differences. Further evaluation using specimens from patients with a spectrum of metabolic copper problems is required to fully validate this concept. The similarity of our data to that of Beetham's,¹⁴ the coincidence of calculated and theoretical means and the fact that the results for patients with Wilson's disease with the lowest ratio would be abnormal using our reference interval is reassuring. The copper:caeruloplasmin ratio may be another useful tool in the diagnosis and investigation of copper metabolism disorders.

Authors' affiliations

Patrick J Twomey, Department of Clinical Biochemistry, The Ipswich Hospital, Ipswich, UK

Adie Viljoen, Department of Clinical Biochemistry, The Lister Hospital, Stevenage, UK

Ivan M House, The Medical Toxicology Unit Laboratory, Guy's & St Thomas' Hospital Trust, London, UK

Timothy M Reynolds, Department of Chemical Pathology, Queen's Hospital, Burton-on-Trent, UK

Anthony S Wierzbicki, Department of Chemical Pathology, St Thomas' Hospital, London, UK

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Correspondence to: Dr P J Twomey, The Ipswich Hospital, Heath Road, Ipswich IP4 5PD, UK; patrick.twomey@ipswichhospital.nhs.uk

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