Reviewer's report

Title: Comparative measurement of CNP and NT-proCNP in human blood samples: a methodological evaluation.

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Reviewer number: 1

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This is a brief manuscript comparing blood processing methods in the evaluation of CNP and NT-proCNP levels. Twelve young men had blood drawn into three tubes, a clotting activator tube, a serum citrate tube and an EDTA tube. For baseline readings, tubes were immediately centrifuged and serum, plasma (Na citrate) and plasma (K EDTA) obtained and assayed.

Tubes also had delayed processing to assess stability. Serum and plasma (Na citrate) were obtained and held at room temperature for 30 minutes and 2 hours. The EDTA tubes were held for the stated time, then centrifuged and plasma obtained.

Samples were assayed for NTproCNP and CNP by a commercial assay.

Results showed NTproCNP levels are not different between processing methods and stable after 30 min 2 hours at room temp.

The CNP results are problematic. Levels were twice as high in the plasma (sodium citrate) than in serum or plasma (K EDTA). The absolute levels are roughly 1,000 fold higher than previously published ranges, done with RIAs. The inter-sample variability is very high with coefficients of variation approaching 50% (compared to about 10% in the reports using RIAs). The authors suggest the difference between the plasma values are likely due to the presence of Na citrate or possibly differences in pH affecting the assay.

The authors try to identify the source of the differences between the CNP RIA and ELISA, but do not show a head-to-head comparison. External standards gave expected results in the ELISA. No conclusion is reached as to the difference.

The authors include a list of studies and reported ranges. They do not mention the 2-fold difference in values for NTproCNP between the Biometric ELISA and the RIA. A head-to-head comparison can be found in Olney RC, Permuy JW, Prickett TCR, Han JC, Espiner EA. 2012 Amino-terminal propeptide of C-type natriuretic peptide (NTproCNP) predicts height velocity in healthy children. Clin Endocrinol. (Oxf). 77(3):416-22 and a more comprehensive adult reference range can be found in Prickett TCR, Olney RC, Cameron VA, Ellis MJ, Richards AM, Espiner EA. Impact of age, phenotype and cardio-renal function on plasma C-type & B-type natriuretic peptide forms in healthy adults. Clin Endocrinol (Oxf). 2012 Sep 11 [Epub ahead of print].
The study is useful only in showing the stability of NTproCNP in processing. Until these questions regarding the CNP ELISA are answered, results using this assay are suspect.

Specific comments:
Methods: an n of only 12 is barely adequate when concluding there is no difference between difference sample groups. A larger n improves confidence that there really is no difference.
Discussion, last sentence: I am not certain what is meant by “disrupting substance.”

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.