Original Article

Stability of creatinine with delayed separation of whole blood and implications for eGFR

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Abstract

Background The stability of creatinine in whole blood is unclear: it is not known if analysis of creatinine in samples with delayed separation could lead to misclassification of chronic kidney disease (CKD) using estimated glomerular filtration rate (eGFR).

Methods Multiple blood samples were taken at a single time-point from five individuals and subject to varying time delays prior to centrifugation, after which serum was separated and analysed for creatinine by five different methods. The effect of time delay on eGFR was further investigated by measuring creatinine on duplicate patient samples arriving in the laboratory after immediate and delayed centrifugation.

Results A significant increase in creatinine was seen by 24 h using kinetic Jaffe methods ($P < 0.025$). Over a period of 31 h creatinine concentration was stable using enzymatic creatinine assays. Using duplicate patient samples, four of 21 patients where specimens were delayed in the laboratory by more than 10 h showed a misclassification of CKD.

Conclusion Delays in sample receipt can lead to significant increases in measured creatinine and misclassification of CKD. Enzymatic creatinine assays are reliable with respect to delayed sample receipt over the time course studied.

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Introduction

Most UK laboratories are following Department of Health advice[1] and reporting estimated glomerular filtration rate (eGFR) on creatinine requests from general practice. These samples in particular can be subject to prolonged transit times, and observations in our laboratory have led to suspicion that delay before centrifugation can lead to falsely increased creatinine concentrations. This has potential implications for eGFR and chronic kidney disease (CKD) classification.

While various studies have indicated stability of creatinine in separated serum,[2,3] stability in whole blood is not clearly defined.[3,4,5] A World Health Organisation document[3] states that creatinine is stable in whole blood for 2–3 days, but another report quotes changes of creatinine in stored blood of approximately 6% per day.[4] This study aimed to investigate the stability of measured creatinine in stored blood using some of the common analytical methods in current widespread use.

Methods

Impact of delay in centrifugation on creatinine concentration

Five healthy non-fasting volunteers (2 male, 3 female, aged 27–64 y) participated in the study for which ethical approval was given. From each subject a set of six 5 mL blood samples were collected into serum separator vacutainers (SST П Advance, BD UK Ltd, Oxford, UK). Samples were kept at room temperature (21 °C) and at defined time points after collection (15 min, 4 h, 8 h, 14 h, 24 h, 31 h) one from each volunteer was centrifuged (1600 *g* for 5 min). After centrifugation samples were stored at 4 °C until analysis. Samples analysed at other laboratories were aliquoted (immediately after

analysis by our own laboratory method) and sent by first class post and refrigerated until analysis.

All samples were analysed for creatinine in singleton in a batch using five different methods, namely; Beckman DXC 800, CRE3 kinetic Jaffe (Beckman Coulter UK Ltd, High Wycombe, UK), Bayer Advia kinetic Jaffe (Bayer Healthcare, Berks, UK), Roche Modular P-800 kinetic Jaffe and Roche Modular P-800 Creatinine Plus enzymatic assay (Roche Diagnostics Ltd, Lewes, East Sussex, UK) and Vitros 5.1 enzymatic creatinine (Ortho-Clinical Diagnostics Ltd, High Wycombe, UK). For all methods the between-batch coefficient of variation (CV %) at a level of 100 umol/L was less than 2%.

Impact of delayed centrifugation on eGFR in patient samples

Twenty-four patients where sample time was stated (10 female, 14 male) and for whom two vacutainers were received by the laboratory were used. Analysis was performed by Jaffe methodology on a Beckman DXC 800 analyser. One of the pair was promptly analysed (within 1 h) after receipt in the laboratory and the second specimen was left at room temperature (21 °C) and analysed after centrifugation up to 28 h later. The eGFR on each sample was determined and the differences between each sample pair were compared.

Statistical analyses were performed using paired t-tests (Analyze-it for Microsoft Excel, Microsoft corporation, Washington USA).

Results

A 24 h delay in centrifugation of whole blood resulted in significant increases in measured creatinine when assayed by each of the individual Jaffe methods investigated ($P < 0.025$). For each Jaffe method, the difference in paired sample creatinine results between 0 h and 24 h expressed as mean difference (95% confidence interval) are as follows: Beckman DXC – 19.7 umol/L (7.3–32.2), Roche Modular – 10.2 umol/L (3.6–16.8), Bayer Advia – 6.2 umol/L (1.5–11.0). The enzymatic methods on both the Roche Modular and Vitros 5.1 analysers showed no significant difference ($P > 0.2$) in creatinine concentration over 31 h (Figure 1).

The most marked effect was observed with our own laboratory method (Beckman DXC 800), with a maximum percentage change in creatinine after 31 h of 67% (Figure 2). Measured creatinine was stable when whole blood was centrifuged within 14 h of collection, on all of the platforms and subjects studied (Figures 1 and 2).

The 24 pairs of patient samples also showed a time dependant increase in measured creatinine using the Beckman DXC 800 Jaffe method (Figure 3). There was no significant difference in creatinine concentration
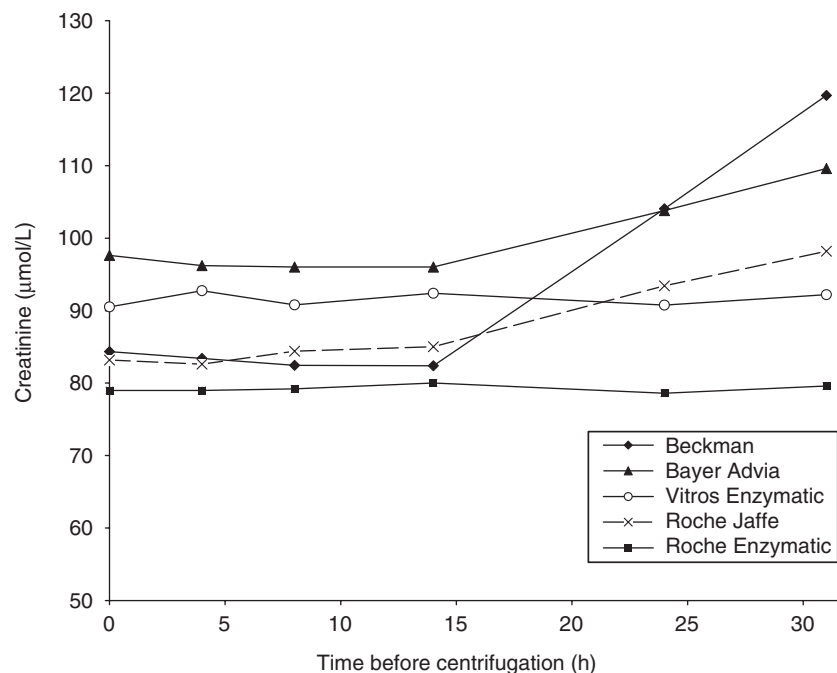

Figure 1 *Mean serum creatinine concentration from five subjects, measured on various platforms (as indicated) after delayed centrifugation of whole blood*
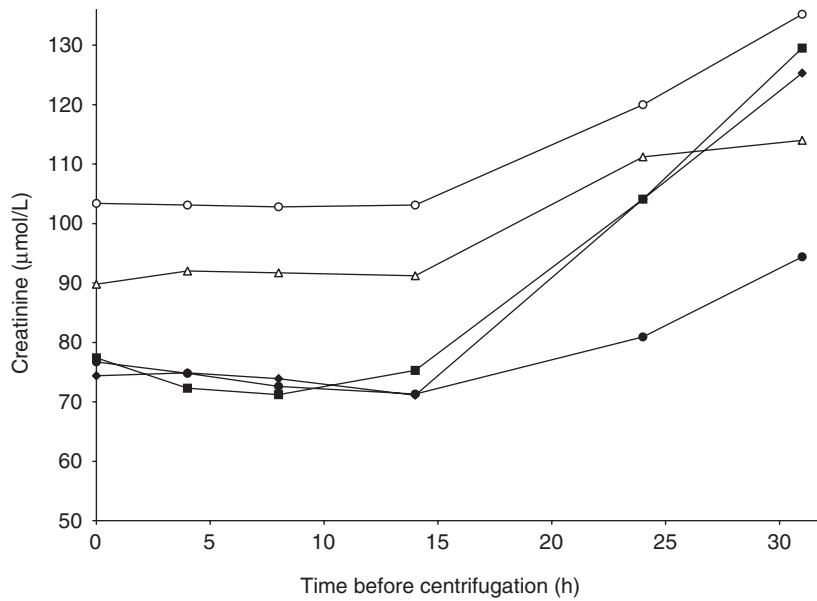
Figure 2   *Creatinine concentration measured in serum after delayed centrifugation of whole blood from five subjects using the Beckman DXC 800 method (open symbols male, closed symbols female)*
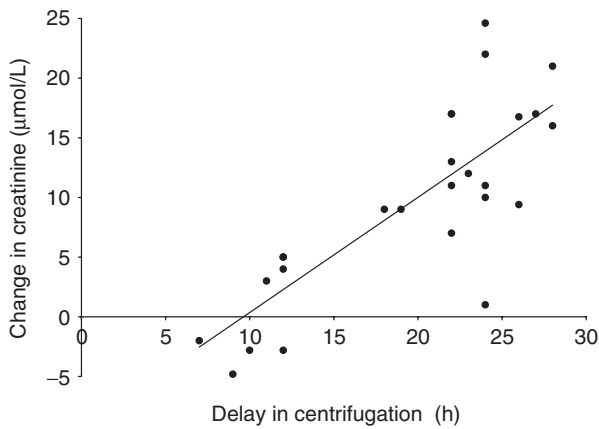

Figure 3   *Difference in patient serum creatinine measured before and after delayed centrifugation using the Beckman DXC 800 method*

before 10 h delay in centrifugation ($P = 0.46$). Significant and variable increases in measured creatinine were seen between 10–24 h delay in centrifugation ($P < 0.001$). In 21 patients where the delay exceeded 10 h, a trend showing significantly decreased eGFR was evident, ($P = <0.001$) resulting in a change in classification of CKD in four of these cases (Figure 4).
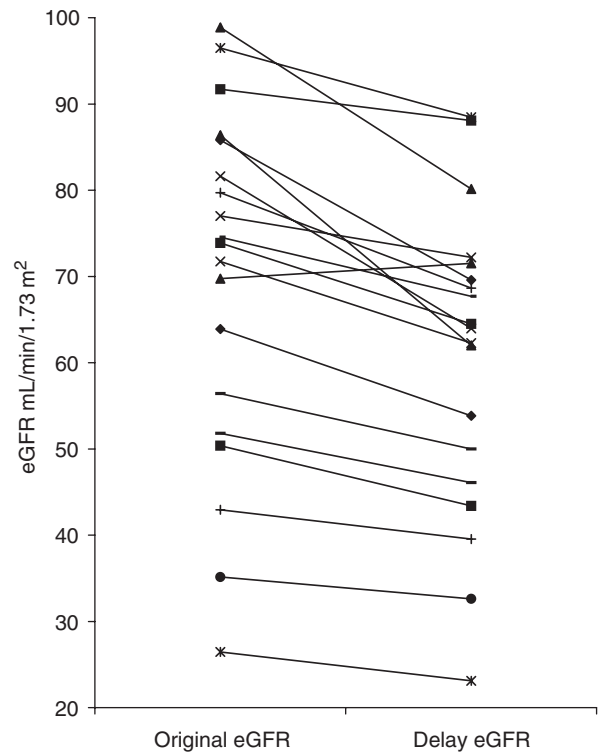
Discussion

The three kinetic Jaffe methods used in this study have revealed an apparent instability of creatinine in whole blood within 24 h. This may have important implica-

Figure 4   *Patient eGFRs (mL/min/1.73m^2) calculated before and after delayed centrifugation (11–28 h) of whole blood (eGFR results exceeding 100 are not shown)*

tions for eGFR and subsequent CKD staging when samples from primary care are delayed in transit. The consistency of results obtained by enzymatic methods within this time frame indicates that this apparent

instability is not due to creatinine itself but to some interfering factor.

The extent of this interference is variable between kinetic Jaffe methods, with the Beckman DXC 800 having an average percentage increase of 42%. The effect on the Bayer Advia and Roche Modular Jaffe methods is somewhat lower, being 12% and 18% respectively after 31 h.

There is variability not only between methods but also between individuals (Figure 2), adding to the uncertainty surrounding the acceptance of delayed samples for creatinine measurement (Beckman DXC800, minimum change 23%, maximum change 67%). As expected, the findings using patient samples corroborate the data from the formal time course study and further demonstrates significant increases in measured creatinine between 10 h and 24 h. The observed change in creatinine impacts on eGFR: whilst some patients with a time delay of between 10-28 h were misclassified in this small study, the individual impact will depend on the proximity of the correct eGFR to a CKD staging threshold.[1] It is noted, however, that the routine reporting of eGFR is not universally recommended for CKD stages 1 and 2,[7] and that only one of the results in the patient group studied falsely transcended the eGFR threshold to CKD stage 3 (Figure 4).

Interference in Jaffe methods is well documented, and compounds such as ketones and pyruvate are known to give notable positive interference with the Beckman DXC 800 method.[6] The build up of such a metabolite *in vitro* could be responsible for the observed effect. In general, Jaffe methods are optimised to minimise interference from commonly encountered interfering substances and variations in the optimisation strategy between manufacturers may be responsible for the method dependent differences seen.

In conclusion, delays in sample receipt can cause significant increases in measured creatinine by commonly used kinetic Jaffe methods. This could lead to misclassification of CKD stage and possibly further unnecessary investigations. The use of enzymatic methods appears to provide more reliable CKD classification in situations where specimens have taken time to reach the laboratory, for example samples from primary care where eGFR is widely applied. It is recommended that time dependant rejection criteria are established in laboratories measuring creatinine using Jaffe methodology.

References

1 Estimating glomerular filtration rate – Information for laboratories. http://www.dh.gov.uk/assetRoot/04/13/30/25/04133025.pdf

2 O'Keane MP, Cunningham SK. Evaluation of three different specimen types for analysis of certain analytes: clinical significance of differences in results and efficiency of use. *Clin Chem Lab Med.* 2006; **44**(5): 662–8

3 *Use of anticoagulants in diagnostic laboratory investigations & stability of blood, plasma, and serum samples.* 2002, WHO/DIL/LAB/99.1 Rev 2. Available at www.eoc.ch/allegati/doc-preanalytica-eolab.pdf

4 Zhang DJ, Elswick RK, Miller WG, Baily LJ. Effect of serum-clot contact contact time on clinical chemistry laboratory results. *Clin Chem* 1998; **44**: 1325–33

5 Clark S, Youngman LD, Palmer A, Parish S, Peto R, Caollins R. *Stability of plasma analytes after delayed separation of whole blood: Implications for epidemiological studies.* Available at http://ije.oxfordjournals.org/cgi/reprint/32/1/125

6 Beckman Coulter. *Chemistry Systems Chemistry information manual [Product No. 962288-AD].* Fullerton. CA: Beckman Coulter, Inc., 2000

7 UK Consensus Conference on Early Chronic Kidney disease. www.rcpe.ac.uk/Whats_New/consensus-statements/final-early-chronic-kidney-disease.pdf