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Lower creatinine concentration values and lower inter-laboratory variation among Swedish hospital laboratories in 2014 compared to 1996: results from the Equalis external quality assessment program

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Abstract

Background: Creatinine measurement for estimation of glomerular filtration rate (GFR) is a frequently used laboratory test. Differences in analytic creatinine methods have caused large inter-laboratory variation. International and national standardization efforts have been made in the last decade.

Methods: This study describes the results of the standardization efforts in Sweden by summarizing data for creatinine concentration in blood plasma in the Equalis quality assessment program during 1996–2014.

Results: Non-compensated Jaffe methods dominated in 1996–2001 (91 of 103 laboratories; 90%) and were then gradually replaced by either compensated Jaffe methods or enzymatic creatinine methods. In 2014 a majority of Swedish hospital laboratories (139 of 159; 87%) used enzymatic methods. The reported mean creatinine value by the Swedish laboratories was about 10 $\mu\text{mol/L}$ higher than the isotope dilution mass spectrometry (IDMS) assured reference value in 2003, but consistent with the reference value from 2009 to 2014. The inter-laboratory CV was 7%–9% for creatinine values until 2007, and thereafter gradually decreased to about 4%–5% in 2014.

Conclusions: The introduction of enzymatic methods in Swedish laboratories has contributed to achieving a low inter-laboratory variation. Also, the reported values are lower for enzymatic methods compared to Jaffe methods, and the values obtained with enzymatic methods were consistent with IDMS certified values established at reference laboratories. Thus, many Swedish hospital laboratories reported 10 $\mu\text{mol/L}$ lower, and more true, creatinine

concentrations in 2012 than in 2003, which may cause bias in longitudinal studies.

Keywords: creatinine methods; external quality assessment; harmonization; inter-laboratory variation; standardization.

Introduction

Creatinine measurement in blood plasma for estimation of glomerular filtration rate (GFR) is one of the most used laboratory tests in health care; around 0.6 creatinine tests per inhabitant and year are performed in Sweden [1, 2]. The dominating creatinine methods are Jaffe or enzymatic methods [3]. There are significant systematic differences between the method groups as well as differences in precision [4]. Jaffe methods are based on creatinine forming a colored product after addition of alkaline picrate. However, the interference by proteins, glucose and substances with a ketone group may cause an overestimation of creatinine, and also reduces the precision of measurements [3, 5]. As the diagnosis of chronic kidney disease may be based on creatinine measurements and subsequent calculations of GFR [6], the frequency of chronic kidney disease may differ depending on which creatinine method laboratories use. Therefore, national and international efforts have been made to standardize and reduce the inter-laboratory variation for creatinine methods during the past decade. In Sweden, Equalis, the national provider of external quality assessment for clinical laboratory investigations, prepared a creatinine free serum in 2003 to assist hospital laboratories in compensating their Jaffe methods to reduce the effects of interfering substances, and reduce the inter-laboratory variation [7]. Additionally, in 2006, the National Kidney Disease Education Program (NKDEP) recommended all manufacturers to standardize their creatinine methods to the high-level isotope dilution mass spectrometry (IDMS) reference method [3]. According to the European *In Vitro* Diagnostics (IVD) directive measurement results should be traceable to standards of higher metrological orders [8]. NKDEP also recommended that a serum reference material with a known creatinine concentration should be developed [3, 9]. The results of the efforts

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in Sweden to harmonize creatinine measurements in line with these recommendations have not been reported.

This study summarizes the reported serum creatinine in the Swedish Equalis program during the years 1996–2014, maps the changes in creatinine methods used by participating laboratories, and describes the inter-laboratory variation.

Materials and methods

External quality assessment program with pooled native serum

Pooled native serum, assumed to be commutable, was sent from Equalis to 112 (mean value; interval 92–159) laboratories 4 to 5 times per year in 1996–2014. The participating laboratories reported their measured creatinine value to Equalis for statistical evaluation. The serum samples were pooled from two donors and purchased from the blood bank at Uppsala University Hospital. One test round in 2014 consisted of minimally processed serum purchased from DEKS prepared from Danish donors. The test material had creatinine concentrations of 58–103 $\mu\text{mol/L}$. Eight test rounds contained serum spiked with creatinine in concentrations between 215 and 692 $\mu\text{mol/L}$. The spiked data are presented separately. The use of donor blood for external quality assessment is approved by the Ethics Committee at Uppsala University. This study does not handle personal data and therefore no additional ethical approval was applied for.

Minimally processed serum test material with IDMS assigned reference value

The test material FHK0108, was sent out by Equalis to hospital laboratories participating in the creatinine program during 13 test rounds

in 2003–2012. It was pooled serum from Danish donors manufactured by DEKS (Danish Institute for External Quality Assurance for Laboratories in Health Care), Herlev, Denmark [10]. The serum was minimally processed and long-term stable at -70 to -80 °C storage. This material had a certified mean value for creatinine of 70.7 $\mu\text{mol/L}$ based on four runs (95% confidence interval 69.9–71.5), assigned by the reference method ID-GC/MS at Ghent University [11].

Statistics

Extreme outliers were detected by visual inspection and obvious input errors were deleted before preparing the statistical reports. The laboratory participants were categorized in method groups according to the information provided by the participating laboratories; enzymatic, Jaffe non-compensated, Jaffe compensated, dry chemistry, i-STAT, and other point of care tests (POCTs). The arithmetic mean was used as the consensus mean. Inter-laboratory variation, in CV%, per calendar year was calculated as the ratio of root mean square error and arithmetic mean value using analysis of variance. Statistics were calculated in Stata 13.0 and Excel 2010.

Results

Creatinine methods

A summary of methods used by the hospital laboratories taking part in external quality assessment in Equalis during 1996–2014 are presented in Figure 1. A majority of laboratories (91 of 103; 90%) used non-compensated Jaffe methods 1996–2001. The original Jaffe methods have then gradually been replaced by either compensated Jaffe methods or enzymatic methods. The proportion of

Figure 1: Methods used for creatinine measurement in hospital laboratories 1996–2014.

In 2012, the manufacturers supplying enzymatic methods were Abbott ($n=11$), Beckman Coulter ($n=12$), Roche ($n=53$), Siemens ($n=26$) and Tokyo Boeki ($n=3$). The manufacturer for dry chemistry was Ortho ($n=21$). The manufacturers for Jaffe were Abbott ($n=1$), Beckman Coulter ($n=4$) and Siemens ($n=3$). i-STAT is manufactured by Abbott ($n=8$) and the other POCT was various manufacturers (Roche, Ortho, Elitech).

laboratories using dry chemistry enzymatic methods have been fairly constant throughout the years; representing 10%–15% of the methods used. In 2014, the majority of laboratories, 139 of 159 (87%), used enzymatic methods including dry chemistry methods.

Minimally processed serum test material with IDMS assigned reference value

Figure 2A shows the mean values of the laboratories reported creatinine for 13 test rounds during 2003–2012. The mean of reported creatinine was about 10 $\mu\text{mol/L}$

higher than the assigned reference value in 2003, about 5 $\mu\text{mol/L}$ higher in 2005–2007 and was consistent with the reference value in 2009–2012. When stratifying the participating laboratories in method groups (Figure 2B), particularly the non-compensated Jaffe methods reported significantly higher values than the reference value in 2003. Also the dry chemistry methods reported high values until the end of 2005. The laboratories using enzymatic and compensated Jaffe methods reported values close to the assigned reference value. Laboratories using the POCT i-STAT started participating 2010 and deviated significantly from the reference value in 2010 and 2012.

Figure 2: Creatinine values for the 13 test rounds from 2003 to 2012.

(A) Mean values and SD of the laboratories' reported creatinine for the 13 test rounds during 2003–2012. The X-axis show which year and week the test round was sent out to the laboratories. Test material is serum FHK0108 with IDMS-assigned mean reference value of 70.7 $\mu\text{mol/L}$ (dashed line), 95% confidence interval 69.9–71.5 (dotted lines). The Y-axis is cut at 60 $\mu\text{mol/L}$. (B) Mean values and 95% confidence interval of the laboratories' reported creatinine for the 13 test rounds, stratified by method group. Test material is serum FHK0108 with IDMS-assigned mean reference value of 70.7 $\mu\text{mol/L}$ (dashed line), 95% confidence interval 69.9–71.5 (dotted lines). The Y-axis is cut at 50 $\mu\text{mol/L}$.

Figure 3: Inter-laboratory CV%.

(A) Inter-laboratory CV in % per calendar year for all participants in the program with native serum. Large dots show results with all point of care tests (POCTs) excluded. Small dots include all methods. The creatinine concentration in the test samples was 58–103 $\mu\text{mol/L}$. (B) Inter-laboratory CV% for each of the eight test rounds with spiked native serum. The creatinine concentrations in the test samples were 215–692 $\mu\text{mol/L}$. Large dots show results with all point of care tests (POCT) excluded. Small dots include all methods.

Inter-laboratory CV for reported creatinine values

The inter-laboratory CV for the reported creatinine values was 7%–9% until 2007, see Figure 3A. Thereafter, the CV gradually decreased reaching 4%–5% in 2014. CV was a few percent higher when including laboratories using i-STAT or other POCT in the analysis. The inter-laboratory CV for the test rounds spiked with creatinine are shown in Figure 3B. The trend is similar at high creatinine concentrations; higher inter-laboratory CV until 2005 (7%) and thereafter gradually decreasing to about 3% in 2014.

Discussion

The study data show that the inter-laboratory dispersion of reported creatinine has continuously decreased since the 1990s, from 7–9 CV% to 4–5 CV% in 2014. The decrease coincides with the successive increase in number of

laboratories using enzymatic methods. Creatinine measured with enzymatic methods was closer to the IDMS-affixed reference value than any other method group in all test rounds indicating a high specificity to creatinine. Enzymatic methods are known to be more specific to creatinine and have higher precision than Jaffe methods and it is desirable to increase the use of these methods in clinical practice [12–15]. It is likely that the high number of Swedish laboratories using enzymatic methods (87%) in 2014 contributes to the present low inter-laboratory variation of 4–5 CV%.

An unacceptably high inter-laboratory variation of 7–8 CV% was seen until 2007 and this is in line with inter-laboratory variations seen in other countries in Europe at that time [4]. A study from 2005 comparing creatinine measurements in commutable serum-based material in 189 laboratories using seven different manufacturers in Belgium, Finland, France, Germany, Italy and the Netherlands showed an inter-laboratory CV of almost 9% at creatinine values within the reference interval [4]. The variation was presumably mainly due to calibration

differences between the manufacturers and this could also be seen in a study comparing creatinine methods from different manufacturers in the US [16]. However, along with efforts to harmonize the creatinine methods on the market, using standard reference material and the reference method IDMS in the calibration process, a clear improvement in overall accuracy of creatinine methods since 2007 can be noticed not only in this Swedish study but also in the US [17] and in European laboratories [12]. Importantly, recent studies have pointed out that high inter-laboratory variability still is an issue and is largely explained by the use of Jaffe methods [15].

Based on the intra- and inter-individual biological variation of creatinine a desirable quality goal of total analytical error has been set to 7.6% by the NKDEP working group [3]. This total error includes the bias due to differences in calibration and the random measurement error including within-laboratory and between-laboratory random variability in local daily calibrations and the analytical imprecision. Equalis' quality goal for creatinine measurements is that 95% of the results in external quality assessment should have a deviation of less than 8% from the consensus mean, or from IDMS derived target value, is close to the desirable goal for total analytical error stated by NKDEP. An optimum quality goal would be a total analytical error of 3.8% according to NKDEP [3]. Reports from Italy [18] and Canada [19] confirm the improvement of the creatinine assays, but that the assays still do not fulfill the desirable performance. In Sweden a large fraction of measurements are done with enzymatic assays, which might explain a better reproducibility of the results than in other countries.

The other main finding in this study is that the mean reported creatinine value by the Swedish laboratories in the quality assessment programs has decreased by about 10 $\mu\text{mol/L}$ during the years 2003–2009. In 2003 most of Swedish laboratories used Jaffe methods. Jaffe methods are known to show false, too high serum creatinine because of interference with proteins, glucose and acetoacetate [3, 5]. The laboratories using Jaffe methods in 2003 significantly overestimated creatinine (mean value 85 $\mu\text{mol/L}$) compared the IDMS-assigned reference value of 71 $\mu\text{mol/L}$. Similar positive bias by Jaffe methods compared to enzymatic methods and the IDMS-method, respectively, have been reported by several research groups [4, 15]. An overestimation by the Jaffe methods of 14 $\mu\text{mol/L}$ corresponds to an underrating of estimated GFR by about 10 mL/min/1.73 m^2 in eGFR, as a mean for the whole patient population. For certain patient groups the use of Jaffe methods possibly led to misclassifications into wrong eGFR-categories [15], unnecessary creatinine monitoring or inappropriate drug dose adjustments.

To assist compensation of the Jaffe methods a creatinine-free serum, containing only interfering substances, was produced by Equalis in 2003. This creatinine-free serum was used by the majority of Swedish laboratories that used Jaffe methods to compensate for the interferences [7]. The mean value for creatinine in the creatinine-free serum was 18 $\mu\text{mol/L}$ (40 laboratories) and the laboratories re-calibrated their Jaffe method accordingly. Also, manufacturers started to provide compensated Jaffe methods with correction of Jaffe results [20]. The significant difference in measured creatinine values in laboratories using compensated and non-compensated Jaffe methods clearly indicates the effect of the introduction of the compensated Jaffe methods. Still, it must be clarified that the compensation of Jaffe methods assumes that the non-creatinine interference is constant, and this is probably not true [14]. The imprecision will still be higher for compensated Jaffe methods than enzymatic methods. Several authors have thus suggested that Jaffe methods for this reason should be abandoned for enzymatic methods because there is still a risk for misclassification of patients or unnecessary monitoring [14, 15]. However, others have suggested that the risk of misclassification by Jaffe methods (i.e. compensated Jaffe methods) is overrated, and will only be critical around the GFR decision limit of 60 mL/min/1.73 m^2 [21]. Also, the higher cost for enzymatic methods must be considered.

Another contributing factor to the decrease in creatinine level during 2003–2009 is obviously the successive increase in number of laboratories changing to enzymatic methods. In 2003, less than 30% of Swedish laboratories used enzymatic creatinine methods whereas in 2009 the enzymatic methods were in majority with 87% of laboratories using them. Enzymatic creatinine methods repeatedly show closer agreement with the IDMS-reference value than other creatinine methods, as confirmed in other studies [2, 4, 15].

The re-calibration and other modifications of the creatinine methods will undoubtedly affect creatinine levels in longitudinal studies with repetitive creatinine measurements. Our results indicate that it is highly likely that the hospital laboratories have changed or modified their creatinine method during the past decade and this may bias the study outcome. Moreover, as creatinine concentration is used for calculation of eGFR [2] and eGFR classifies and stages a patient for the diagnosis of kidney disease [6] recalibrations of creatinine methods may bias the frequency of chronic kidney disease over time [15].

With the exception for the minimal processed serum (FHK0108), the commutability of the materials used for this study was only assumed and not tested. The FHK0108 was produced according to same carefully

described procedures [10] as were the materials used for the Nordic Reference Interval Project, and for which the materials were found to be commutable for creatinine, among other constituents [22]. The reasons for assuming commutability also for the other specimens are that the presently used sample handling and storage are generally associated with a high likelihood for commutability [23]. The samples were pooled, minimally processed, native serum, and the aliquots were frozen at -70°C . However, it cannot be excluded that differences in creatinine concentrations between method groups could in part be related to matrix-related bias in some of the test materials [24].

In conclusion, the extensive introduction of enzymatic methods in Swedish hospital laboratories has contributed to the achievement of reaching the creatinine quality goal of a low inter-laboratory variability. Further, many Swedish hospital laboratories report more than $10\text{ }\mu\text{mol/L}$ lower creatinine concentrations in 2012 than 2003 which may affect patient care, as well as introducing a bias in longitudinal studies.

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