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Evaluation of Dade Behring N Latex Cystatin C Reagent on Abbott Ci8200

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ABSTRACT

Plasma cystatin C has been shown in several studies to be superior to plasma creatinine for estimation of glomerular filtration rate (GFR). Reporting cystatin C results in mL/min using conversion formulas for transforming cystatin C expressed as mg/L to GFR expressed as mL/min has greatly facilitated the clinical use of the marker. At our hospital we have an increasing demand for cystatin C and at present we perform over 1,400 cystatin C analyses a month. The test is available at all hours. This in combination with the volume emphasises the need to have the assay close to the routine chemistry instrument to reduce handling time per test and time to report test results. We have thus evaluated the Dade Behring N Latex Cystatin C assay (Dade Behring, Deerfield, IL, USA) on Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA). The nephelometric method on the ProSpec (Dade Behring) and the turbidimetric method on Architect ci8200 showed very good agreement ($y = 1.0072x + 0.0042$; $R^2 = 0.987$). Accordingly, running the cystatin C analyses on a chemistry instrument (Architect ci8200) would be proper to increase the availability of the analysis and reduce turnaround times.

INTRODUCTION

In the last decades, serum or plasma creatinine has become the most commonly used marker of glomerular filtration rate (GFR) (1,2). Despite the common use, creatinine has limitations as marker for renal function. GFR is often calculated from plasma creatinine using the Cockcroft-Gault (3) or the Modification of Diet in Renal Disease (MDRD) study equations (4). These equations consist of several parts, which makes them susceptible to errors especially if the calculation is performed manually. Creatinine is influenced by factors such as age, gender, muscle mass, physical activity and diet (5). It is also insensitive for detecting small decreases in GFR, in the so-called creatinine-blind GFR area, due to the non-linear relationship between plasma creatinine concentration and GFR (6). Thus, there is a need for better GFR markers. Our laboratory has been using plasma cystatin C as a marker for GFR since 3 years.

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Cystatin C is a polypeptide with a molecular mass of 13 kDa and a probable ellipsoid shape with axes of about 30 and 45 Å (7). A recent meta-analysis has indicated that cystatin C is superior to plasma creatinine (8) as a marker of renal function. One remaining problem in the use of cystatin C as a GFR marker is the lack of a procedure to transform cystatin C concentrations in mg/L to GFR values in mL/min. To provide clinicians with reliable and readily available GFR data based on single plasma measurements of cystatin C, we report cystatin C both in mg/L and as a "cystatin C calculated GFR" in mL/min since 3 years. This in combination with a 24 h availability of cystatin C has led to a very rapid increase in cystatin C requests during the last 3 year-period. At present we perform over 1,400 cystatin C analyses and there is still a continuous increase. To meet this increasing demand we wanted to move the test from the ProSpec to the Architect ci8200. The aim of this study was to compare the results obtained with the nephelometric assay performed on ProSpec with the nephelometric assay on Architect ci8200 using the same reagent to see if we reliably could move the assay from one instrument to the other.

MATERIALS AND METHODS

Patient samples and assays

The comparison was performed with 202 consecutive routine requests for cystatin C analysis. Plasma cystatin C measurements were first performed by latex enhanced reagent (N Latex Cystatin C, Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring) and calibrators from Dade Behring. The test was performed according to the recommendation of the manufacturer. The total analytical imprecision of the method was 4.8 % at 0.56 mg/L and 3.7% at 2.85 mg/L. The results from the instrument was then used to calculate and report a "cystatin C calculated GFR" using the formula $y = 77.24x^{-1.2623}$(9). The study was approved by the local ethical board at Uppsala University (01-167).

Plasma cystatin C measurements on Architect ci8200 was performed using the following instrument settings: Primary wavelength 572 nm, sample blank and spline calibration method. 145 mL reagent 1 (3 mL supplement reagent (Dade Behring) and 42 mL diluent (Dade Behring)) was mixed with 20 µL reagent 2 (undiluted N Latex Cystatin C, Dade Behring) and 15 µL sample diluted 1:50 with Dade Behring diluent. The sample dilution was performed automatically by the instrument.

Statistical calculations

Statistical analysis was performed utilizing Excel 2000 (Microsoft Corporation, Seattle, WA, USA).

RESULTS

Imprecision data for the Architect ci8200

The total imprecision of the instrument was analyzed at 0.96 mg/L (CV 4.19%; n=22) and 1.96 mg/L (CV 5.43%; n=22). The imprecision was also tested by running 80 sam-

ples in duplicate. The CV for these duplicate samples was 3.98% (mean 1.48 mg/L; range 0.58-3.84 mg/L).

Correlation between Cystatin C analyzed on ProSpec and Architect ci8200
Cystatin C values analyzed on both instruments showed strong agreement (R2 = 0.987, Fig. 1). The linear regression analysis showed a slope very close to 1.00 and

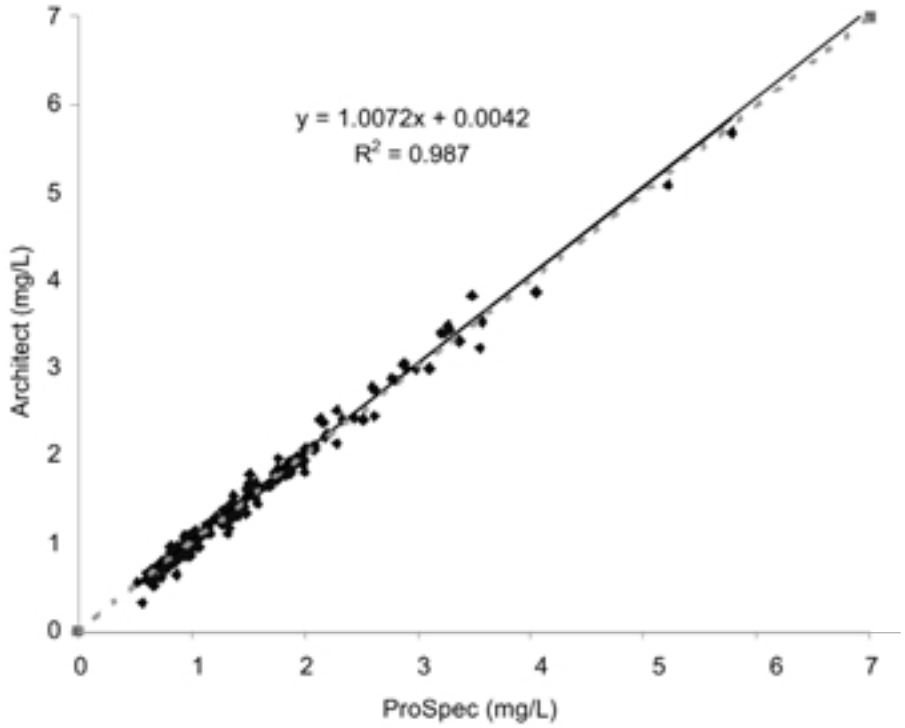

Fig 1. Correlation between cystatin C in 202 patient samples analyzed on a ProSpec (x-axis) and Architect ci8200 (y-axis). The filled line indicates the linear correlation between the two methods and the dotted line indicates the 45º angle.

an intercept close to 0 (y = 1.0072x + 0.0042). The bias plot (Fig. 2) displayed a good agreement between the two methods within the studied range. Hemolytic, icteric and lipemic samples were included in the comparison. The results from these samples also showed good agreement between the two methods.

DISCUSSION

Glomerular filtration rate (GFR) is generally accepted as the best overall index of renal function and is an important marker for renal disease. Reduced GFR influences the metabolism and clearance of many pharmaceuticals used today. Thus, in many cases the recommended dose has to be adjusted depending on the patient´s GFR. For

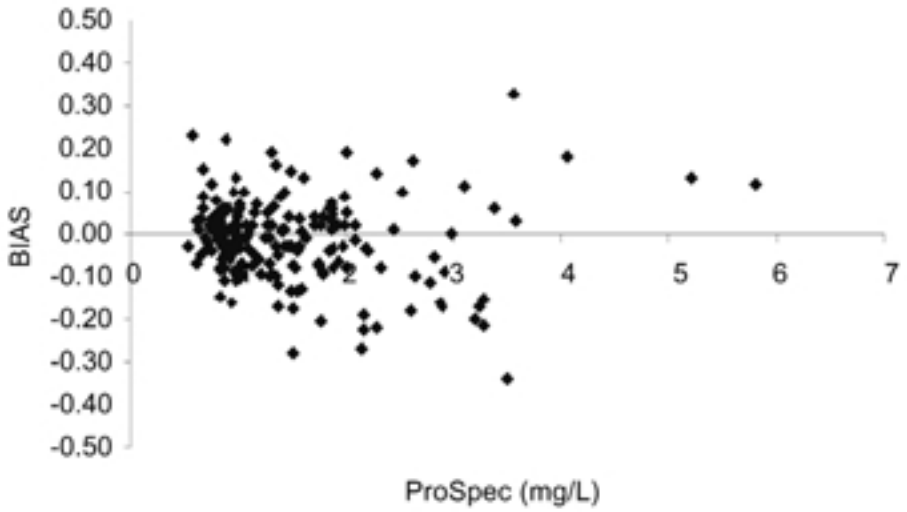

Fig. 2. Bias plot for cystatin C analyzed on a ProSpec and Architect ci8200 (n=202).

instance, antibiotics and cytotoxic drugs are usually prescribed according to GFR. There is thus a need for GFR markers. Inulin, Iohexol and ^{51}Cr-EDTA clearances are considered the golden standards for GFR measurements. The disadvantage with these assays is that they are cumbersome, costly and slow which may delay the start of treatment. Assays such as plasma creatinine and cystatin C can provide rapid test results. Creatinine often overestimates GFR in patients with slight reductions in GFR. It is also difficult to evaluate creatinine in elderly patients with low muscle mass. These patients may have creatinine values in their normal range due to the combination of low muscle mass and reduced GFR.

In Sweden, calculation of GFR, using the Cockcroft-Gault equation (3), is generally performed manually in the wards. Such manual calculations are time consuming and therefore costly. This led to the development of formulas to automatically convert cystatin C in mg/L to a calculated GFR in mL/min (9,10). To increase the quality of GFR measurements our hospital has tried to replace plasma creatinine measurements with plasma cystatin C for patients that require a more exact GFR quantification e.g. for prescribing pharmaceuticals that are eliminated through the kidneys. The rapidly increasing cystatin C volumes illustrate the clinical value of the assay. We also expect an increased use of cystatin C as a risk marker for cardiovascular disease and mortality in the future (11,12). To meet this demand it would be advantageous to move the assay to a chemistry instrument to improve the handling of the samples. We here report the first method using Dade Behring reagents on a chemistry instrument (Architect ci8200). The close correlation between the two assays shows that the same formula for calcula-

tion of GFR in mL/min can be used.

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