Evaluation of NGAL Test™, a fully-automated neutrophil gelatinase-associated lipocalin (NGAL) immunoassay on Beckman Coulter AU 5822

Giuseppe Lippi1,*, Rosalia Aloe1, Antonietta Storelli2, Gianfranco Cervellin2 and Tommaso Trenti3
1 U.O. Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy
2 U.O. Pronto Soccorso e Medicina d’Urgenza, Dipartimento di Emergenza-Urgenza, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy
3 Dipartimento di Patologia Clinica, AUSL di Modena, Modena, Italy

Abstract

Background: The neutrophil gelatinase associated lipocalin (NGAL) has been identified as the most promising biomarker of acute kidney injury (AKI). This study was aimed to evaluate a NGAL immunoassay on Beckman Coulter AU 5822.

Methods: NGAL Test™ (BioPorto Diagnostics A/S) is a particle-enhanced turbidimetric immunoassay. The within- and between-run imprecision were assessed on three urine samples. The linearity was assessed by serially diluting two urine samples with low and high NGAL concentration. The comparison study was performed with Abbott ARCHITECT NGAL, on 70 urine samples.

Results: The within-run imprecision was comprised between 1.0% and 2.3%, whereas the between-run imprecision was between 1.2% and 2.0%. The linearity was excellent in the range between 18 ng/mL and 790 ng/mL (r=1.000; p<0.001). A highly significant agreement was observed between NGAL Test™ on Beckman Coulter AU5822 and Abbott ARCHITECT NGAL (r=0.925; p<0.001), although the method exhibited a bias of +65%. Excellent sensitivity and specificity against the ARCHITECT values were found at 200 ng/mL.

Conclusions: This analytical evaluation attests that the NGAL Test™ has several technical and analytical advantages, including no manual pretreatment, low volume of sample (i.e., 3 µL), fast turnaround time (approx. 10 min), low imprecision, wide dynamic range, optimal linearity.

Keywords: acute kidney failure; creatinine; evaluation; neutrophil gelatinase-associated lipocalin (NGAL).

Introduction

Acute kidney injury (AKI), formerly known as acute renal failure is a major public health problem worldwide and results in a substantial increase of hospital stay and death. As regards to the general population, the incidence of less severe AKI and AKI treated with renal replacement therapy span approximately from 2000 to 3000 and from 200 to 300 per million population per year, respectively (1). In intensive care units (ICU) the incidence ranges from 1% to 25% according to the different definition of AKI, with an overall mortality rate as high as 60% (2, 3). Up to 60% of patients might also develop AKI during the ICU stay, with a greater risk of longer permanence in the ICU, increased in-hospital death and dialysis dependence.

The historical diagnostic criteria of AKI have been traditionally based on acute changes in serum creatinine or urine output. The serial measurement of serum creatinine, although remaining the biochemical gold standard for chronic renal failure (4), carries however several caveats in the setting of AKI, due to variations of the reference range according to age, gender, diet, drugs, muscle mass, and the poor specificity in the presence of prerenal azotemia. Moreover, secretion accounts for 10%–40% of creatinine clearance, thereby masking a decrease in glomerular filtration rate. The sensitivity of creatinine testing is poor in the presence of adequate renal reserve, because a creatine increase (or an estimated glomerular filtration rate decrease) might be masked by a compensatory improved functionality of the kidney, so that serum creatinine would only become abnormal when more than 50% of glomerular filtration rate is lost (5, 6). The diagnostic performance of the urine output is also dramatically low, due to the low sensitivity (i.e., several AKI patients do not develop oliguria) and specificity (i.e., several oliguric patients do not develop AKI). Moreover, several drugs, such as diuretics and vasopressors are potential confounders (5).

A variety of urinary and serum proteins have been recently investigated as potential early and sensitive markers of AKI. Among these, neutrophil gelatinase associated lipocalin (NGAL) was found to have the strongest body of clinical evidence so far (5, 7, 8). NGAL is a relatively small (i.e., 25 kDa) protein belonging to the lipocalin family, which was originally discovered in 2003 by a genome-wide analysis of
kidney genes that are induced in response to experimental AKI in animals (9). The greatest clinical advantage of NGAL is that the modification of this marker in the setting of AKI occurs at least 24–48 h earlier as compared with serum creatinine or urine output. A recent systematic review and meta-analysis reported an NGAL area under the curve (AUC) in receiver operating characteristics (ROC) curve of 0.815 (95% CI 0.732–0.892) across all AKI settings, with specificity and sensitivity of 85% and 76%, respectively (10). The prediction using urinary NGAL also showed a better trend than plasma/serum NGAL.

The widespread introduction of NGAL assessment in clinical practice is however hampered by the limited availability of commercial techniques, which currently comprises only manual enzyme linked immunoassays (ELISAs), a urinary fully-automated immunoassay developed for a single analytical platform (i.e., ARCHITECT® analyzer; Abbott Diagnostics), and a whole blood point of care immunoassay (Triage® NGAL Device; Biosite-Inverness Medical) (11). Therefore, the aim of this study was the evaluation of an NGAL immunoassay, which has been licensed for use on a variety of clinical chemistry analyzers. The method has been proposed for plasma and urine samples, but the current evaluation was limited to urine, since this biological matrix is deemed preferable for both clinical and analytical perspectives (10).

Materials and methods

Method description

The NGAL Test™ (BioPorto Diagnostics A/S, Gentofte, Denmark, which is distributed in Italy by Sentinel Diagnostics, Milan, Italy) is a particle-enhanced turbidimetric immunoassay for the quantitative determination of NGAL in human urine and EDTA plasma, on a variety of automated clinical chemistry analyzers, including Roche Modular and Cobas series, Siemens Advia series, Beckman Coulter AU series and Abbott Architect c8000. The text is a development of a previously exiting manual ELISA commercialized by the same company. This turbidimetric fixed time assay typically uses 3 µL of sample, 50 µL antibody and 150 µL of reaction buffer. Briefly, after a short incubation the reaction is started by the addition of an immunoparticle suspension (polystyrene microparticles coated with mouse monoclonal antibodies to NGAL). The presence of NGAL in the sample causes the immunoparticles to aggregate. The degree of aggregation is quantified by the amount of light scattering measured as absorption of light at 700 nm. The NGAL concentration in the sample is finally calculated by interpolation on an established calibration curve and the assay is completed within 10 min. The on-board stability of reagents is reported to be 4 weeks at 2–8°C. The linearity is reported to span between 25 ng/mL and 5000 ng/mL. The reference range of this assay, as stated by the manufacturer, is 0.7–9.8 ng/mL in urine. The manufacturer has also determined that the NGAL concentration in an isolated urine specimen should exceed 250 ng/mL to indicate the presence of renal disorder, including AKI. The NGAL Test™ is currently pending FDA clearance for diagnostics use in the US. The analytical performance of this immunoassay was assessed on the novel Beckman Coulter clinical chemistry platform AU5822 (Beckman Coulter Inc., Brea, CA, USA).

Imprecision studies

The imprecision studies were carried out on three urine samples with low (approx. 200 ng/mL), intermediate (approx. 600 ng/mL) and high (approx. 800 ng/mL) NGAL concentrations. Both within- and between-run imprecision were assessed in 20 sequential runs (i.e., 20 consecutive days for between-run imprecision), by using an identical reagent lot and calibration curve. Final results were expressed in terms of coefficient of variation (CV).

Linearity

A patient urine with high NGAL concentration (approx. 800 ng/mL) was serially diluted at fixed ratios (1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; 9:1) with an additional urine sample displaying low NGAL concentration (approx. 20 ng/mL), to cover the most clinically significant range of concentrations. Serial dilutions were analyzed in duplicate and the theoretical values were calculated from the measured values of the undiluted specimens. Linearity was assessed with calculation of linear regression analysis and Spearman’s correlation coefficient (r).

Comparison study

The comparison study was performed on 70 consecutive patient’s urine randomly selected from those referred from the emergency department for urgent urinalysis and displaying a broad range of NGAL values. All samples were tested within 2 h from collection. Results of NGAL on AU5822 were compared with those obtained on the same urinary samples on the Abbott ARCHITECT NGAL, whose technical features and analytical performances are reported elsewhere (12), and which has been previously reported to be the best NGAL immunoassay for detecting AKI (11). The mean percentage bias and the 95% confidence interval (CI) were estimated by Bland-Altman plot analysis. The analytical accuracy, evaluated against results on paired specimens, was also assessed by ROC analysis.

Statistics

The statistical evaluation was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK). The study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

Results

The within- and between-run imprecision of the NGAL is shown in Table 1. The within-run imprecision was comprised between 1.0% and 2.3%, whereas the between-run imprecision was comprised between 1.2% and 2.0%, thereby showing comparable or even better performance than the Abbott ARCHITECT NGAL (CVs comprised between 2.1% and 5.3% in one study, and between 1.7% and 5.7% in another) (11, 12), but a much better performance than the Biosite Triage (CVs comprised between 5% and 16%) (11). The linearity of NGAL Test™ on Beckman Coulter AU5822 was excellent in the range between 18 ng/mL and 790 ng/mL, as mirrored by the equation of the linear regression analysis plotted against theoretical NGAL concentration (y=1.00x–5.6; r=1.000; p<0.001). The results of the comparison study
are shown in Figures 1 and 2. A highly significant agreement was observed between NGAL Test™ on Beckman Coulter AU5822 and Abbott ARCHITECT NGAL (NGAL Test™=1.46 x Abbott ARCHITECT NGAL +6.9; r=0.925; p<0.001) (Figure 1). The mean bias calculated with Bland-Altman plot analysis was 65% (95% CI, from 49% to 81%) (Figure 2). Finally, the analytical accuracy of NGAL Test™ on Beckman Coulter AU5822, as assessed against the values of Abbott ARCHITECT NGAL, yielded an AUC of 0.993 (p<0.001). As regards the definition of the optimal threshold value, excellent sensitivity (100%) and specificity (98%) plotted against Abbott ARCHITECT NGAL values (i.e., cut-off 159 ng/mL) were found at a diagnostic threshold of 200 ng/mL, thereby at a slightly lower value than that declared by the manufacturer (i.e., 250 ng/mL).

**Discussion**

NGAL is a host defence protein which is normally present in secondary granules of human neutrophils, but it is also expressed at a low level in other tissues including the kidney, prostate and epithelia of the respiratory and alimentary tracts, in adenomas and inflamed epithelia of the bowel, adenocarcinomas of the breast and urothelial carcinomas (13). The levels of this biomarker rise rapidly after acute renal injury, so that it can be reliably used for predicting AKI in a variety of clinical settings including intensive care, emergency medicine, renal transplantation and procedures involving the use of intravenous contrast media and other known nephrotoxic agents. Testing for urinary NGAL in patients with suspected AKI is also associated with lower costs as compared with current diagnostic methods (14).

According to the results of this evaluation, the NGAL Test™ immunoassay carries several technical and analytical advantages, including no need of manual pretreatment steps, a low volume of sample required (i.e., 3 µL vs. 150 µL with Abbott ARCHITECT), a very fast turnaround time (the assay is completed within 10 min vs. 35 min on Abbott ARCHITECT, and over 2 h with manual ELISAs), a high throughput enabled by its application to the vast majority of automated clinical chemistry analyzers from manufacturers such as Roche, Abbott, Siemens and Beckman Coulter, a very low imprecision especially when compared with previously commercialized NGAL methods, a wide dynamic range and an optimal linearity in the range comprising the most clinically significant diagnostic values (i.e., between 18 ng/mL and 790 ng/mL).

Although this test has been developed on either serum/plasma or urine, reliable evidence from the current scientific literature suggests the latter biological matrix seems preferable for analytical and clinical reasons. These include the higher delta of concentration after AKI (e.g., in urine NGAL shows a 10,000-fold concentration rise to the highest levels, whereas
in plasma the maximum rise is about 100-fold), in serum or plasma the diagnostic efficiency of this marker for predicting AKI is decreased due to the major contribution of extrarenal sources (i.e., AUC of 0.775 in serum/plasma vs. 0.837 in urine) (5, 10). This was also confirmed in a recent study which showed that that urinary NGAL is a better biomarker for chronic renal diseases in children than serum NGAL (15).

Conflict of interest statement

Authors‘ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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References