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Analytical validation of Gentian NGAL particle-enhanced enhanced turbidimetric immunoassay (PETIA)

Gian Luca Salvagno[a,b,*], Anna Ferrari[b], Matteo Gelati[a], Giorgio Brocco[b], Giuseppe Lippi[a,b]

[a] Section of Clinical Biochemistry, University of Verona, Verona, Italy
[b] Laboratory of Clinical Chemistry and Hematology, University Hospital of Verona, Verona, Italy

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ABSTRACT

Objectives: This study was designed to validate the analytical performance of the new Gentian particle-enhanced enhanced turbidimetric immunoassay (PETIA) for measuring neutrophil gelatinase-associated lipocalin (NGAL) in serum samples.
Design and methods: Analytical validation of the Gentian NGAL assay was carried out on a Roche Cobas c501 and was based on assessment of limit of blank (LOB), limit of detection (LOD), functional sensitivity, imprecision, linearity and concordance with the BioPorto NGAL test.
Results: The LOB and LOD of Gentian NGAL were found to be 3.8 ng/mL and 6.3 ng/mL, respectively. An analytical coefficient of variation (CV) of 20% corresponded to a NGAL value of 10 ng/mL. The intra-assay and inter-assay imprecision (CV) was between 0.4 and 5.2% and 0.6 and 7.1% and the total imprecision (CV) was 3.7%. The linearity was optimal at NGAL concentrations between 37 and 1420 ng/mL ($r = 1.00$; $p < 0.001$). An excellent correlation was observed between values measured with Gentian NGAL and BioPorto NGAL in 74 routine serum samples ($r = 0.993$). The mean percentage bias of the Gentian assay versus the Bioporto assay was +3.1% (95% CI, +1.6% to +4.5%).
Conclusions: These results show that Gentian NGAL may be a viable option to other commercial immunoassays for both routine and urgent assessment of serum NGAL.

1. Introduction

The neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, is a single disulfide-bridged protein of 178 aminoacids with a molecular mass of 22 kDa, increasing to 25 kDa after glycosylation [1]. The protein can be detected in both serum and urine in three main molecular forms; a 25-kD monomer, a 45-kD disulfide-linked homodimer and a 135-kD heterodimer, in which the protein is covalently linked to matrix metalloproteinase 9 (MMP-9) by means of a single intermolecular disulfide bridge [1]. Although NGAL was originally detected in activated neutrophils, its expression was later identified in many other tissues and organs including the kidney, lungs, intestine and prostate. More recently, the ectopic expression of NGAL has been reported in patients with various malignancies such as kidney, colorectal, gastric, pancreatobiliary, hepatic, breast, gynecological and hematologic cancers [2].

The clinical use of NGAL has gained attention since 2003, when it was convincingly shown that the concentration of this biomarker increases considerably immediately after acute kidney injury (AKI) [3]. Since then, several studies have confirmed that

* Corresponding author at: Section of Clinical Biochemistry, University of Verona, Verona, Italy.
E-mail address: gianluca.salvagno@univr.it (G.L. Salvagno).

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NGAL may be considered as an early and reliable biomarker of AKI in various clinical settings [4–7].

Ideally, a candidate biomarker of AKI should have many important characteristics, such as being measurable with a noninvasive, easy, rapid and inexpensive technique and displaying high sensitivity and specificity for AKI. It should also permit early detection of AKI, provide information about severity and reversibility of disease, and predict response to treatment and clinical outcome [8]. Among the various potential biomarkers of AKI, which also include kidney injury molecule-1 (KIM-1), N-acetyl-β-d-glucosaminidase (NAG), liver fatty acid-binding protein (L-FAB), and interleukin-18 (IL-8), NGAL has been shown to be the most promising, and is the only one for which fully-automated assays have become available to date [8]. The availability of quick and reliable automated immunoassays allows rapid laboratory assessment and timely communication of test results to clinicians, enabling early diagnosis of AKI and reliable prediction of outcome. The measurement of NGAL in serum or plasma is also considered to be more convenient than measurement in urine, since urine output may be considerably reduced in patients with AKI. The reliability of urine measurement is also challenged by several preanalytical issues such as the poor standardization of urine collection procedures compared to those for venous blood and the possible contamination with leukocytes in patients with urinary infections, which may ultimately decrease the diagnostic efficiency of this biomarker [9]. The clinical significance of measuring NGAL has also been recently highlighted in conditions other then AKI, such as acute bacterial meningitis [10] and bacterial peritonitis [11]. Notably, a growing number of studies have reported that NGAL may be used as an efficient biomarker for early diagnosis and predicting the clinical outcome of certain types of cancer [12–14].

Therefore, the present study was designed to validate the analytical performance of Gentian NGAL, a new fully-automatable immunoassay for measuring NGAL in human serum and urine.

2. Materials and methods

2.1. Assay characteristics

Gentian NGAL (Gentian, Moss, Norway) is a particle-enhanced enhanced turbidimetric immunoassay (PETIA), which can be applied to a vast array of automated clinical chemistry analyzers. According to the assay principle, urine or plasma samples are mixed with a solution containing anti-NGAL immunoparticles. The NGAL present in the sample aggregates with the anti-NGAL immunoparticles to generate immunocomplexes, which increase the turbidity of the solution. Light absorbance, measured between 480 and 560 nm, is directly proportional to the concentration of the molecule in the test sample. The NGAL concentration is calculated form a 6-point pre-diluted standard calibration curve, with calibrators values comprised between 0 and 3168 ng/mL. The manufacturers' literature states that the calibrators contain NGAL of recombinant origin, with values assigned according to Gentian's internal value transfer protocol, based on published methods [15]. In this study, the method has been applied for use on a Roche Cobas c501 module (Roche Diagnostics, Indianapolis, IN, USA). The quoted NGAL serum/plasma reference range is 13.7–104.6 ng/mL, the total sample volume needed for the assay is 3 μL and test results are available in 10 min.

2.2. Analytical performance

The analytical validation of Gentian NGAL PETIA consisted of calculation of limit of blank (LOB), limit of detection (LOD) and functional sensitivity, and estimation of inter-assay and intra-assay imprecision, linearity, and correlation with another widely used NGAL PETIA (BioPorto Diagnostics).

The calculation of the LOB and LOD was carried out as described by Armbruster and Pry [16], as follows: [LOB] = mean value + 1.645* standard deviation (SD) of 10 consecutive replicates of saline; [LOD] = LOB + 1.645* SD of 10 replicates of a routine serum sample with the lowest measurable NGAL concentration. The functional sensitivity of the assay was defined as the lowest NGAL concentration which could be measured with an imprecision (CV) ≤ 20%. This value was determined by performing nine serial dilutions in sample buffer (i.e., from 1:2 to 1:512) of a routine serum sample with NGAL concentration of 1485 ng/mL. Each dilution was measured in 10 replicates and the imprecision was calculated for each dilution. A model fit was constructed to extrapolate the NGAL value associated with a CV of 20%.

The imprecision studies were performed using three serum pools selected for low (~40 ng/mL), intermediate (~260 ng/mL) and high (~1465 ng/mL) NGAL concentrations. Within-and between-run imprecision was respectively assessed in 20 consecutive runs and over 10 consecutive working days, using the same calibration curve and identical reagent lots. Results were reported as coefficient of variation (CV%). The total analytical imprecision was calculated according to the formula proposed by Krouwer and Rabinowitz [17].

The linearity of the assay was tested as follows: a routine serum sample with high NGAL concentration (1420 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 another routine serum sample with a low NGAL concentration (37 ng/mL), to produce samples with a clinically significant range of NGAL concentrations in serum. The serial dilutions were then measured in duplicate and theoretical values were calculated from the measured values of undiluted specimens.

2.3. Method comparison

The comparison study was performed using routine serum samples referred from the emergency department to the laboratory for assessment of serum creatinine, over one working morning. All samples were tested within 2 h of collection. The results of the Gentian NGAL assay were compared with those obtained on the same sample with BioPorto NGAL Test (BioPorto Diagnostics A/S,