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A comparison of three commercial platforms for urinary NGAL in critically ill adults

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Abstract

Background: Early biomarkers for acute kidney injury (AKI) diagnosis are needed since an increase in serum creatinine levels is a late marker. Neutrophil gelatinase-associated lipocalin (NGAL) is one of the most promising AKI biomarkers. Prior to routine clinical use, it is necessary to evaluate and validate a high-throughput commercially available method for NGAL detection. The aim of this study was to do an independent validation and comparison of the analytical performance of three different commercially available urine NGAL (uNGAL) assays.

Methods: Urine samples (n=110) were obtained from various patient groups with and without AKI. All urine samples were processed using Architect NGAL assay, Siemens Advia® 2400 NGAL test, and Siemens Dimension Vista® NGAL Test™, based on the three different platforms.

Results: Overall, there was good agreement among the three assays: Spearman's rank correlation coefficient between Architect and Vista was 0.989 (95% confidence interval [CI], 0.983–0.993), between Architect and Advia, 0.962 (95% CI, 0.937–0.977), between Vista and Advia 2400, 0.975 (95% CI, 0.961–0.984). We observed a negative bias of Architect compared with the other assays: comparing Architect to Vista, the mean bias was –55.7 ng/mL (95% CI, –74.3 to –37.0 ng/mL); comparing Architect to Advia 2400, the mean bias was –40.9 ng/mL (95% CI, –56.4 to –25.4 ng/nL). The bias is proportional to the concentration of uNGAL and is more pronounced at higher levels, while irrelevant near the tested cutoff levels of 100 and 190 ng/mL. Comparing Vista and Advia 2400, the mean bias was 10.1 ng/mL (95% CI, 1.5–18.8 ng/mL). Intra-assay imprecision was generally acceptable across all assays; coefficient of variation ranged from 0.8% to 5.3%.

Conclusions: All three methods for uNGAL showed acceptable performance for the tested parameters and are comparable with each other at clinically relevant cutoffs. However, Architect yields lower results than the other two methods, with a bias more pronounced at higher uNGAL concentrations, suggesting additional standardization efforts will likely be necessary to better harmonize the uNGAL methods at various clinically relevant cutoffs.

Keywords: acute kidney injury (AKI); diagnosis; method comparison; neutrophil gelatinase-associated lipocalin (NGAL).

Introduction

Acute kidney injury (AKI) is defined as a common syndrome that results from numerous causative factors and occurs in many clinical settings, with different clinical manifestations, extending from a minimal change in serum creatinine to anuric renal failure. AKI represents an important and under-recognized problem, with adverse immediate and long-term consequences. In current clinical practice, AKI is typically diagnosed by measuring acute changes in serum creatinine. Unfortunately, creatinine is a delayed and unreliable indicator during acute changes in kidney function [1–5].

Over the past few years, innovative and emerging proteomic technology has revealed new hope for identification of novel biomarkers to improve risk stratification, inform clinical decisions, diagnose AKI, and guide therapy. In recent years, many potential markers, such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1, cystatin C, liver fatty-acid binding protein, were identified and many studies were published to validate the utility of these markers in AKI [6].

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So far, the most extensively studied AKI marker is NGAL, which can be measured in both blood and urine [4, 7–14]. However, reference ranges, adjusted for age, gender, and ethnicity, as well as reliable cutoff values for ruling in and ruling out AKI, are still lacking. Technical differences across biomarker assays, including issues of reliability and accuracy, pose additional challenges for the practicing clinician looking for specific threshold values. The ADQI Consensus Conference on AKI Biomarkers highlighted that set cutoffs for various biomarkers will be in part determined by regulatory intended use guidelines, platform standardization, and inter-laboratory calibration, as has been done for the natriuretic peptides in heart failure and serum creatinine for estimation of glomerular filtration rate [15, 16].

Several commercially available NGAL assays are proposed in the last years, first as research-use-only enzyme-linked immunoassays, and more recently, also for clinical use, Conformité Européenne (CE) Market in vitro diagnostics, applied on automatic chemistry analysers. A number of studies have evaluated the analytical characteristics of various NGAL assays [17–21], and new methods are continuously launched in the market (http://www.labmark.cz/soubory/bioporto-aplikace-ngal-test-on-siemens-dimension-vista.pdf). A recent meta-analysis suggested that standardized platforms had better diagnostic accuracy than research-based assays [10]. Furthermore, standardized platforms have many technical and analytical advantages, such as the avoidance of manual pretreatment, low volume of sample need for the test, and the fast turnaround time. The first automated standardized platform for NGAL was Architect from Abbott Laboratories (Abbott Park, IL, USA). The Architect NGAL assay is a chemiluminescent microparticle non-competitive two-site sandwich immunoassay that utilizes two mouse antibodies recognizing distinct NGAL epitopes. More recently, BioPorto Diagnostics came up with CE-approved assays, first with 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 (Siemens, Beckman Coulter, Abbott, and Roche), and later with a particle-enhanced nephelometric channel immunoassay, on Siemens Dimension Vista® Integrated Chemistry System. An essential step in the transition of this biomarker to routine clinical and research use is to determine whether these different assays give comparable results or require further harmonization and standardization at predicated clinically relevant cut-points.

The aim of this study was to do an independent validation and comparison of the analytical performance of these three commercially available urine NGAL (uNGAL) assays, based on the three different platforms.

Materials and methods

Patient samples

Urine samples (n=110) were obtained from various patient groups with and without AKI. Patients with documented AKI were recruited from the general medical-surgical intensive care unit (ICU) of St. Bortolo Hospital: AKI stage 1–2 not requiring renal replacement therapy (RRT) (n=10) and septic patients with AKI stage 3 requiring RRT (n=81). AKI was defined according to Kidney Disease Improving Global Outcomes AKI criteria [22], and sepsis was defined according to consensus guidelines [23]. Individuals without AKI were recruited from two populations: non-septic ICU patients without AKI (n=10) and healthy volunteers with no known illnesses (n=10). Urine was collected from spontaneous voids (healthy volunteers) or from indwelling Foley catheters. The institutional review board approved the protocols for recruitment and sample collection, according with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. Urine samples were centrifuged for 10 min at 1500 rpm within 30 min of collection, and supernatants were aliquoted and stored at –70 °C. Assays were performed after a maximum of two freeze-thaw cycles. All testing was carried out according to manufacturers' specifications.

NGAL assays

All urine samples were processed using three different assays (Table 1): the Architect NGAL assay (Abbott Laboratories, North Chicago, IL, USA) utilizes a non-competitive, sandwich format with chemiluminescent signal detection. The two mouse anti-NGAL antibodies are directed against distinct, non-overlapping NGAL epitopes. The calibrators are prepared with recombinant human NGAL expressed and purified at Abbott Laboratories. The recombinant NGAL is a full-length protein. The assay calibrators are at 0, 10, 100, 500, 1000, and 1500 ng/mL, and the concentration of NGAL measured is proportional to the signal. In terms of imprecision, declared within run coefficient of variations (CV) by the manufacturer was 4.4% for low control (20 ng/mL) and 2.2% for high control (1174 ng/mL). The measuring interval of the Architect urine NGAL assay is 10–1500 ng/mL. Values <10 and >1500 ng/mL were represented as 10 and 1500 ng/mL, respectively, for all analyses.

The Siemens Advia® 2400 NGAL Test is a particle-enhanced turbidimetric assay (BioPorto Diagnostics, Gentofte, Denmark). Polystyrene microparticles are coated with murine monoclonal antibodies against NGAL. The NGAL antigen causes the aggregation of the microparticles. Increased turbidity is measured by light adsorption. The assay calibrators (REF ST002CA) are at 0, 50, 150, 600, 1500, and 3000 ng/mL, and the concentration of NGAL measured is proportional to the signal. Manufacturer-declared limit of quantification (LOQ) is 25 ng/mL, and the measuring range is 25–3000 ng/mL. For precision, declared CV is 2.4% for low control (196.1 ng/mL) and 1.4% for high control (489.1 ng/mL). Values <25 and >3000 ng/mL were represented as 25 and 3000 ng/mL, respectively, for all analyses.

Table 1: Description of three automated uNGAL assays, as declared by manufacturers.

	Chemiluminescent	Turbidimetric	Nephelometric
Type of antibody	Mouse	Mouse	Mouse
Reporting range, ng/mL	10–1500	25–3000	25–3000
Within-run imprecision (low control)			
CV, %	4.4	2.4	1.7
Value, ng/mL	20	196	216
Within-run imprecision (high control)			
CV, %	2.2	1.4	1.8
Value, ng/mL	1174	489	554
Manufacturer	Abbott Laboratories (North Chicago, IL, USA)	BioPorto Diagnostics (Gentofte, Denmark)	BioPorto Diagnostics (Gentofte, Denmark)
Platform	Architect system (Abbott, Abbott Park, IL, USA)	Siemens Advia 2400 (Siemens Healthcare Diagnostics, Newark, DE, USA)	Siemens Dimension Vista (Siemens Healthcare Diagnostics, Newark, DE, USA)
Product	Abbott Diagnostics Division (Lisnamuck, Longford, Ireland)	BioPorto Diagnostics (Gentofte, Denmark)	BioPorto Diagnostics (Gentofte, Denmark)

The Siemens Dimension Vista NGAL Test™ is a particle-enhanced nephelometric immunoassay (BioPorto Diagnostics). The NGAL antigen causes the aggregation of the microparticles, and increased turbidity is measured by 90° light scatter. The assay calibrators (REF ST002RA) are at 20, 150, 600, 1500, and 3000 ng/mL, and the concentration of NGAL measured is proportional to the signal. Declared LOQ is 25 ng/mL, and the measuring range is 25–3000 ng/mL. For precision, declared CV is 1.7% for low control (215.6 ng/mL) and 1.8% for high control (554.4 ng/mL). Values <25 and >3000 ng/mL were represented as 25 and 3000 ng/mL, respectively, for all analyses.

Precision

To verify the declared precision of the three methods, we utilized procedures specified in Clinical and Laboratory Standards Institute protocol EP5-A2 [24, 25]. For the Siemens Advia 2400 NGAL Test™ and Siemens Dimension Vista NGAL Test, two sets of controls (low and high) were run in duplicate, twice a day (testing days×run×replicate: 20×2×2, on two levels, total period=22 days, n=80 per control). These controls were ready-to-use samples of recombinant human NGAL in a 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid buffer (NGAL Test™ Control Kit, ref ST003CA; BioPorto Diagnostics) supplied by the manufacturer; the assigned value for the used control lot (no. 062042) was 198 ng/mL for low level and 488 ng/mL for high level. For Architect NGAL assay, we did not perform these procedures; instead, we refer to data from a recent publication [19]. Inter- and intra-assay CVs of ≤15% and ≤10%, respectively, were considered to be acceptable [20].

Statistical analysis

uNGAL concentrations of all samples were compared across the three assays using modified Bland-Altman plot and Passing-Bablok analysis. The mean bias, slopes, intercepts, and corresponding 95% confidence intervals (CIs) were estimated. Method comparison studies were also performed, in which the Architect assay was used as the comparison method and analytical concordance was assessed. We evaluated this at two specific cutoff values for uNGAL, which have been suggested in the literature: at 100 ng/mL [26–28] and at 190 ng/mL [10].

All statistical analysis was performed using the Medcalc® (MedCalc Software bvba, Ostend, Belgium) Software package, and SPSS 20 (SPSS, Chicago, IL, USA) software packages, with two-sided $p<0.05$ considered as statistically significant.

Results

A total of 110 urine samples were analyzed using the three commercially available uNGAL assays. A scatterplot of the distribution of uNGAL levels is shown in Figure 1. For all three assays, values were lowest for control subjects (median, 24.2 ng/mL, 95% CI, 10.0–38.1, interquartile range (IQR), 10.0, 35.8, for Architect; median, 42.4 ng/mL, 95% CI, 29.3–49.5, IQR, 34.1 , 45.7, for Advia 2400; median, 38.9 ng/mL, 95% CI, 30.3–48.7, IQR, 33.1, 47.3, for Dimension Vista) and for non-septic ICU patients without AKI (median, 19.4 ng/mL, 95% CI, 13.8–41.6, IQR, 16.5, 37.3, for Architect; median, 27.4 ng/mL, 95% CI, 25.0–111.0, IQR, 25.0, 79.0, for Advia 2400; median, 38.6 ng/mL, 95% CI, 33.9–71.2, IQR, 35.5, 64.0, for Dimension Vista). uNGAL concentrations were highest for septic ICU patients with AKI stage 3 (median, 784.3 ng/mL, 95% CI, 668.6–1166.5, IQR, 498.6, 150,] for Architect; median, 835.9 ng/mL, 95% CI, 709.1–1289.6, IQR, 505.2, 1809.6, for Advia 2400; median, 898.9 ng/mL, 95% CI, 747.6–1525.2, IQR, 519.3, 1968.9, for Dimension Vista), and intermediate for ICU

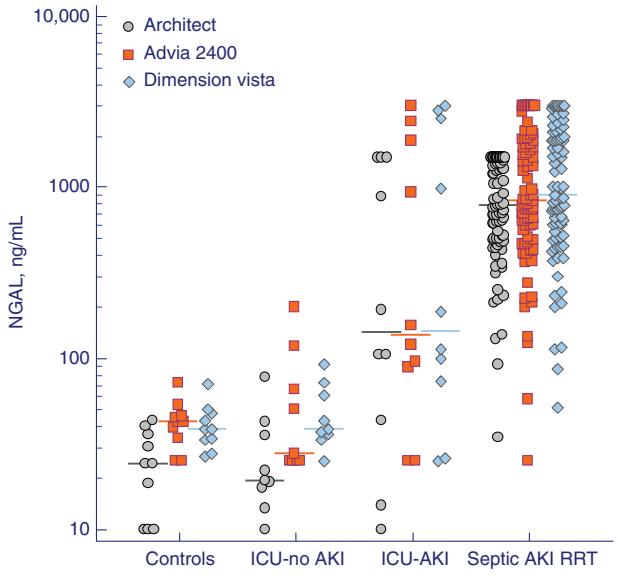

Figure 1: Scatterplot of uNGAL concentrations using the three assays in control subjects, non-septic ICU patients without AKI, ICU patients with AKI stage 1–2, and septic ICU patients with AKI stage 3 requiring RRT.
Horizontal lines indicate the median for each group.

patients with stage 1–2 AKI (median, 147.8 ng/mL, 95% CI, 28.0–1500, IQR, 43.8, 1500.0, for Architect; median, 138.4 ng/mL, 95% CI, 55.5–2154.4, IQR, 89.3, 1866.2, for Advia 2400; 150.4 ng/mL, 95% CI, 48.9–2678.7, IQR, 73.9, 2535.5, for Dimension Vista).

Figure 2 shows the correlations among the three assays. As expected from the difference in the upper limits of the measuring range between the assays, there is a scatter of values for Vista (Figure 2A) and Advia 2400 (Figure 2B) at a value of 1500 ng/mL for Architect when all specimens are included. When restricting the analyses to samples with values ≤1500 ng/mL for Vista and Advia 2400 (n=75) samples, the statistical parameters demonstrate good agreement between Architect and Vista (Figure 2D, Spearman's rank correlation coefficient=0.989, 95% CI, 0.983–0.993, p<0.001), with a least squares linear regression line Vista=1.13 (Architect)+6.94 (95% CI for slope and intercept, 1.08–1.17 and 0.86–13.99, respectively).

Similarly, there was good agreement between Architect and Advia (Figure 2E, Spearman's rank correlation coefficient=0.975, 95% CI, 0.961–0.984, p<0.001) with a least squares linear regression line, Advia=1.10 (Architect)+3.60 (95% CI for slope and intercept, 1.06–1.14 and –5.45 to 10.96, respectively).

When comparing Advia with Vista, a tight correlation was seen up to values of approximately 1000 ng/mL; above these values, results tended to be slightly lower with Advia 2400 except for four specimens, for which results with Advia were markedly lower than with Vista (Figure 2C). When limiting the analyses to samples with uNGAL concentrations ≤1500 ng/mL for Vista and Advia 2400, agreement was good between the two methods (Figure 2F, Spearman's rank correlation coefficient=0.978, 95% CI, 0.965–0.986, p<0.001) with a least squares linear regression line Advia=0.97 (Vista)+2.79 (95% CI for slope and intercept, 0.95–0.99 and –3.92.38 to 8.62, respectively).

The agreement between methods, evaluated in terms of Bland-Altman plot analysis for samples with values ≤1500 ng/mL (Figure 3), showed a negative bias of Architect compared with both BioPorto assays, which is also proportional to the concentration levels of NGAL (the negative bias is constant in percentage). When comparing Architect with Vista (Figure 3A), the mean bias was –55.7 ng/mL (95% CI, –74.3 to –37.0 ng/mL). Agreement limits ranges from –214.3 ng/dL (95% CI, –246.3 to –182.3 ng/mL) to 103.0 (95% CI, 71.0–134.9 ng/mL). The bias is proportional to the concentration of uNGAL and is more pronounced at higher levels, while irrelevant near the cutoff levels. Comparing Architect and Advia 2400 (Figure 3B); the mean bias was –45.5 ng/mL (95% CI, –62.6 to –28.5 ng/nL); agreement limits ranges from –190.9 ng/dL (95% CI, –220.8 to –161.6 ng/mL) to 99.8 (95% CI, 70.5–129.1 ng/mL).

Comparing Vista and Advia 2400, the mean bias was minimal 10.1 ng/mL (95% CI, 1.5–18.8 ng/mL). Also, the agreement limits were reduced, ranging from –63.4 ng/mL (95% CI, –78.2 to –48.6 ng/mL) to 83.6 ng/mL (95% CI, 68.8–98.4 ng/mL). The agreement remains quite constant along the concentration levels range; there was a small positive bias for samples over 1000 ng/mL. Intra-assay imprecision was generally acceptable across all assays (Table 2). For the Architect NGAL assay, reported total CVs ranged from 3.9% to 5.3% for the low-concentration control samples and from 2.2% to 3.0% for the high-concentration sample [19]. The imprecision was slightly better for the Advia 2400 NGAL Test, with CV ranging from 1.4 to 2.7 at the low concentration and from 0.8 to 1.8 at high concentration. The CVs for Dimension Vista NGAL Test ranged from 1.4% to 3.6% for the low control level and from 2.1% to 3.3% for the high control.

At the posited cutoff of 100 ng/mL, analytical concordance data showed that Dimension Vista as having 98.8% agreement with Architect with 0 false-positive and 1 false-negative result (Table 3). Advia 2400 had 96.5% agreement with Architect 2 false-positive and 1 false-negative results, and 97.6% agreement with Dimension Vista. κ Values were 0.971, 0.912, and 0.942 for Dimension Vista

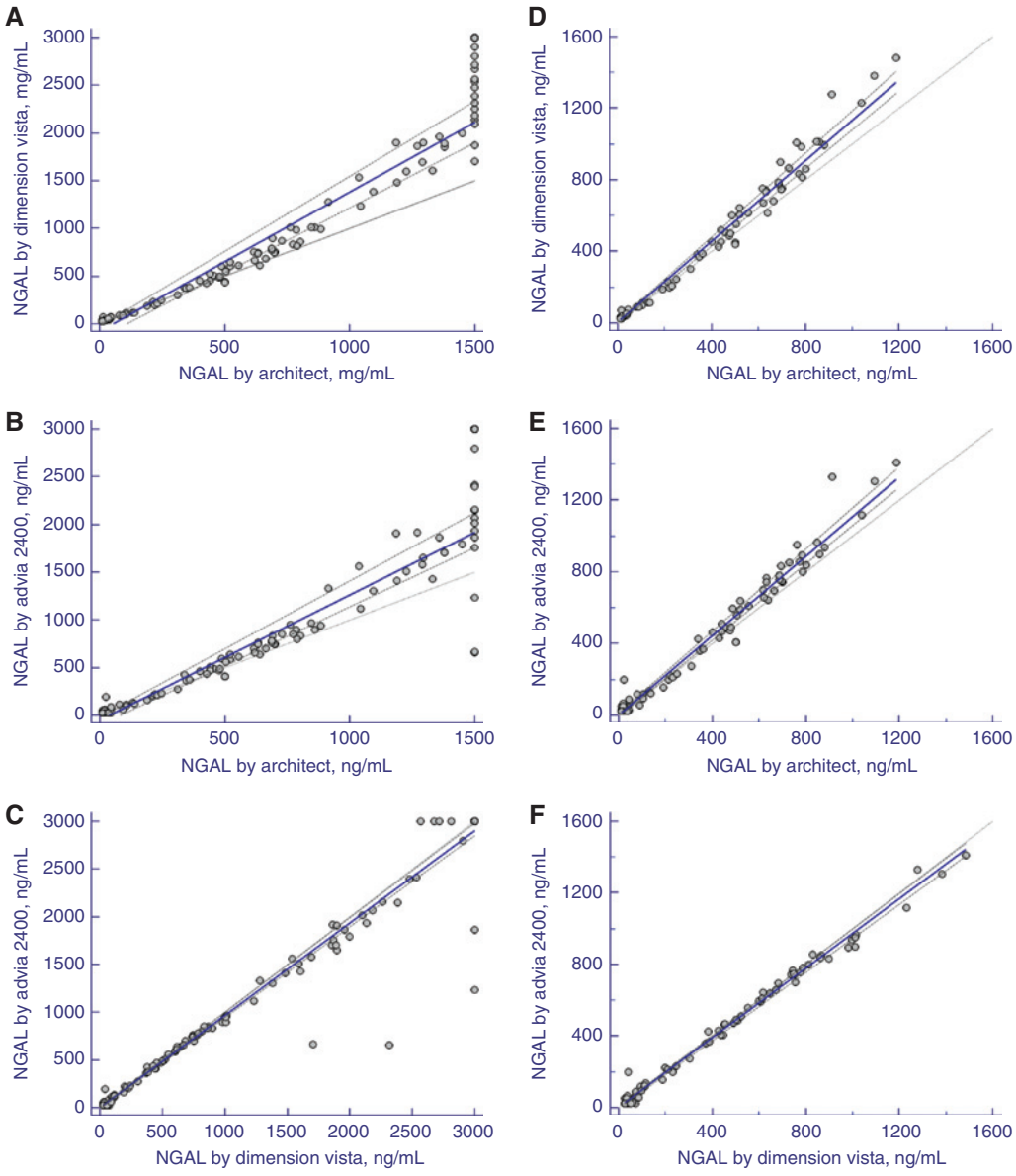

Figure 2: Linear regression analysis of uNGAL concentrations.
Using all (n=110) values: (A) Architect and Vista, (B) Architect and Advia 2400, and (C) Vista and Advia 2400; using ≤1500 ng/mL (n=75): (D) Architect and Vista, (E) Architect and Advia 2400, and (F) Vista and Advia 2400.

and Architect, Advia 2400 and Architect, and Advia 2400 and Dimension Vista, respectively ($p<0.001$ for all).

At the second cutoff of 190 ng/mL, similar results were seen (Table 4). Dimension Vista showed a 98.8% agreement with Architect, while Advia 2400 had 97.6% agreement with Architect, and 98.8% agreement with Dimension Vista. κ Values were 0.973, 0.945, and 0.973 between Dimension Vista and Architect, Advia 2400 and Architect, and Advia 2400 and Dimension Vista, respectively ($p<0.001$ for all).

In a limited sample of four patients, serial urine samples were collected over 2–8 days. We plotted these serial measurements of uNGAL. On the average, lower values were seen with Architect compared to the two Bio-Porto assays, but we observed parallel trends in all uNGAL three assays (Figure 4).

Discussion

The past decade has brought important advances in the identification and validation of novel blood and urine biomarkers for AKI. Among the multitude of biomarkers, a few, notably cystatin C and NGAL, are measurable in both

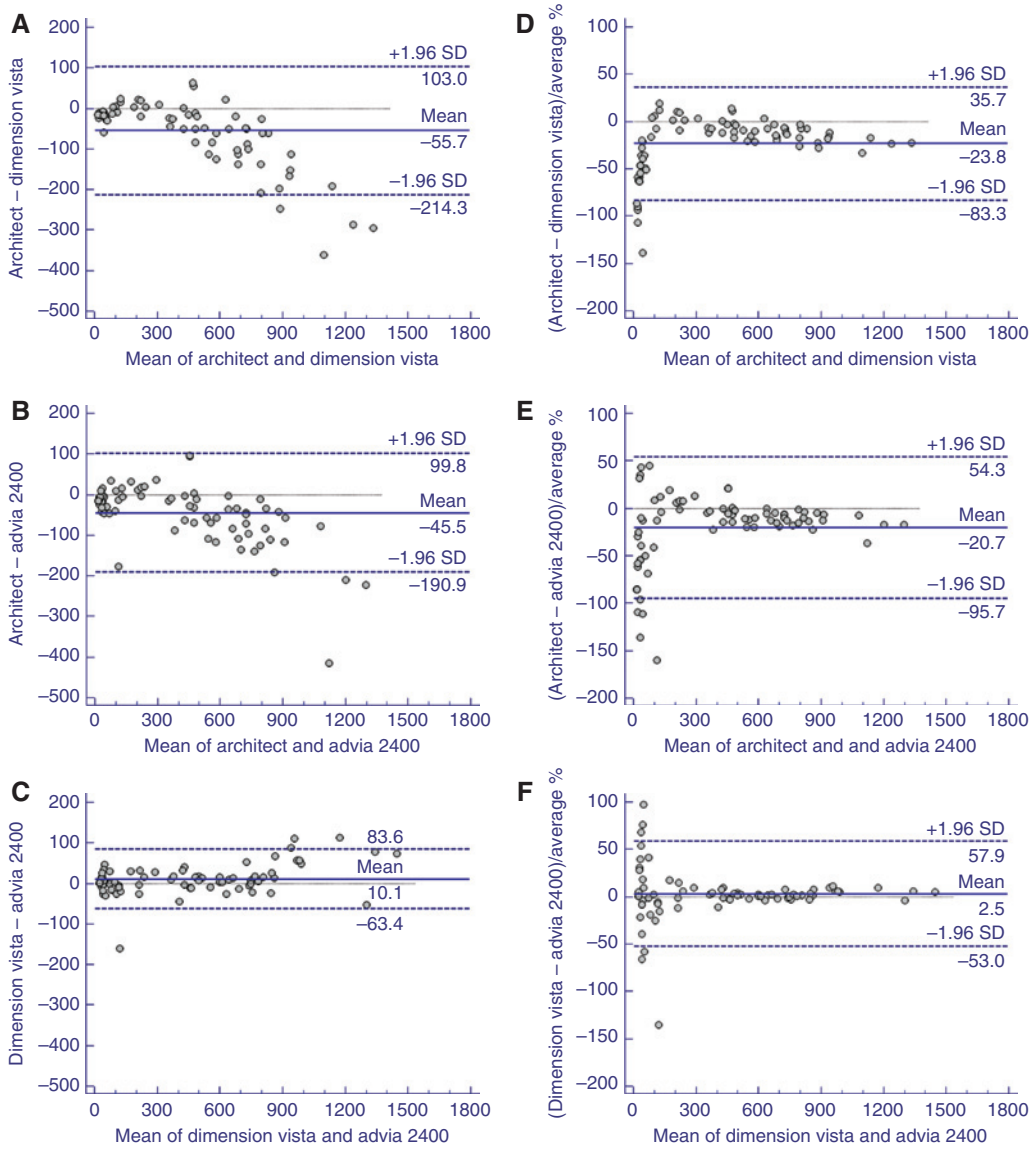

Figure 3: Bland-Altman plots of uNGAL concentrations.
(A) Architect and Vista, (B) Architect and Advia 2400, and (C) Vista and Advia 2400.

blood and urine using assays that are commercially available for clinical use in certain countries. A large number of clinical studies have suggested that NGAL should be considered a useful diagnostic and prognostic biomarker for AKI [7, 10, 15, 27, 29]. However, there are a number of challenges for its adaptation into clinical practice, including technical differences across biomarker assays and issues of reliability and accuracy.

A meta-analysis of 19 studies showed that standardized platforms for NGAL measurement performed better than research-based assays [10]. Such techniques also have the advantage of higher throughput and better reproducibility. Since the introduction of the first commercial immunoassay Architect in 2008 [28], the NGAL turbidimetric immunoassay has been licensed for use on a number of chemistry autoanalyzers, including the Advia 1800, the Advia 2400, and Beckman Coulter AU 5822 [20, 21]. The application for the Advia 1800 platform has since then been withdrawn [20]. More recently, the nephelometric assay for use on autoanalyzers including the Dimension Vista has also been approved for use in Europe. An essential prerequisite prior to widespread research and clinical use is independent validation and comparison of analytical performance of available assays, to ensure interpretability across these tests and enable valid inter-study comparisons.

We compared three commercially available uNGAL assays for laboratory chemistry analyzer platforms, based

Table 2: Summary of imprecision data for three automated uNGAL assays (between-run precision).

Run	Replicate, n=20	Low control		High control	
		Mean, ng/mL	Total CV, %	Mean, ng/mL	Total CV, %
Architect NGAL assay[a]					
Run 1	Repl1	19.9	4.9	1185.3	2.5
	Repl2	19.7	3.9	1170.9	2.3
Run 2	Repl1	19.3	5.3	1215.8	3.0
	Repl2	19.7	4.5	1184.4	2.2
Siemens Advia 2400 NGAL Test					
Run 1	Repl1	206.6	1.4	509.8	0.8
	Repl2	209.7	2.5	508.9	1.7
Run 2	Repl1	209.7	1.5	508.9	1.3
	Repl2	207.8	2.7	511.0	1.8
Siemens Dimension Vista NGAL Test					
Run 1	Repl1	195.3	1.4	484.7	2.6
	Repl2	198.6	2.2	479.5	2.1
Run 2	Repl1	196.1	3.2	483.9	2.9
	Repl2	198.1	3.6	483.9	3.3

CV, coefficient of variation; Repl, replicate. [a]Data for Architect from Reference [19].

Table 3: Analytic concordance of three automated uNGAL methods at 100 ng/mL.

			Architect
	≥100 ng/mL	<100 ng/mL	Total
Dimension Vista			
≥100 ng/mL	85	0	85
<100 ng/mL	1	24	25
Total	86	24	
			Architect
	≥100 ng/mL	<100 ng/mL	Total
Advia 2400			
≥100 ng/mL	85	2	87
<100 ng/mL	1	22	23
Total	86	24	
			Advia 2400
	≥100 ng/mL	<100 ng/mL	Total
Dimension Vista			
≥100 ng/mL	85	0	85
<100 ng/mL	2	23	25
Total	87	23	

Cutoff of 100 ng/mL was obtained from References [26–28].

Table 4: Analytic concordance of three automated uNGAL methods at 190 ng/mL.

			Architect
	≥190 ng/mL	<190 ng/mL	Total
Dimension Vista			
≥190 ng/mL	81	0	81
<190 ng/mL	1	28	29
Total	82	28	
			Architect
	≥190 ng/mL	<190 ng/mL	Total
Advia 2400			
≥190 ng/mL	81	1	82
<190 ng/mL	1	27	26
Total	82	28	
			Advia 2400
	≥190 ng/mL	<190 ng/mL	Total
Dimension Vista			
≥190 ng/mL	81	0	81
<190 ng/mL	1	28	29
Total	82	28	

Cutoff of 190 ng/mL was obtained from Reference [10], in which cutoff was 190.2 ng/mL for AKI across all settings and 193.2 ng/mL for AKI prediction using uNGAL.

on three different methods (Table 1) in terms of a number of important aspects. First, we found a good correlation at uNGAL concentrations ≤1500 ng/mL among the three assays, with inter-assay Spearman's correlation coefficients ranging from 0.961 to 0.986 and slopes on least square linear regression ranging from 0.94 to 1.16.

The limit of 1500 ng/mL was determined by the upper limit of measurement, which for Architect is 1500 ng/mL, while for Dimension Vista and Advia 2400 is 3000 ng/mL. We have avoided repeating the measures diluting the samples, considering a value >1500 ng/mL high anyway The best correlation was between Architect and Vista (R=0.989 and slope=1.13). Second, the Architect tended to have slightly lower numerical results compared to both of the BioPorto assays, with a mean bias of –55.7 ng/mL. This bias appeared to be more pronounced at higher uNGAL concentrations. This was also noted on a prior evaluation of the NGAL test on the Beckman Coulter AU 5822 [21]. This may indicate slight differences in calibration and/or in assay design. Of note, this bias was more evident at values well above potential clinical decision values [10, 27, 28].

Comparing Dimension Vista and Advia 2400, three discordant samples were observed (Figure 2C), for UNGAL concentrations over 1500 ng/mL measured by Dimension Vista. One of them was a patients with previous chronic kidney disease (CKD) stage 2; the second became anuric 12 h later; the third, an extracorporeal membrane

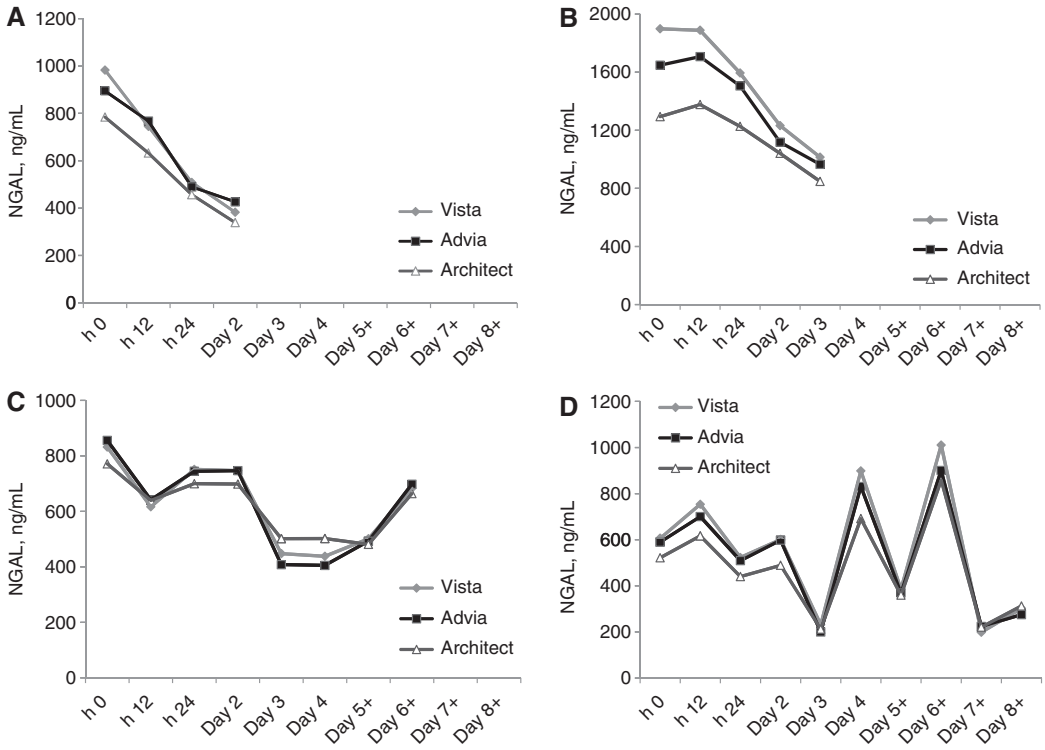

Figure 4: Serial uNGAL measurements in four patients using three automated uNGAL assays.

oxygenation-treated patient, died the day after. It could be possible that a previous CKD and/or a decreasing excretion of urine could affect the sensitivity of assays methods, which is higher in the nephelometric method. Also if "outliers", these results do not change the clinical classification at the proposed cutoff of 100 or 190 ng/mL.

In a limited sample of four patients, we also observed parallel trends in all uNGAL three assays on serial monitoring. However, the very small number of patients with serial values precludes any definitive conclusions on this aspect.

Overall, precision was acceptable for all three assays (Table 2). We evaluated the imprecision for the Advia 2400 and Dimension Vista assays using control levels near the decision levels, i.e. with an NGAL declared concentration of 198 and 488 ng/mL, while for the Architect assay, we refer to recently published data [19]. The new nephelometric method, applied on Dimension Vista, showed an imprecision level similar to that specified by the manufacturer (Table 1) and comparable to that of Architect and other prior studies [19–21, 30]. More imprecision was observed with the turbidimetric method on Advia 2400, both for a bias in the target values and in the CV%, particularly in run 2 (Table 2). However, inter- and intra-assay CVs of ≤15% and ≤10% could be considered acceptable for clinical purposes [20], and all CVs of the three assays were within this range. Finally, we also evaluated the concordance of the three assays at two cutoffs, which have been suggested in the literature. A uNGAL cutoff value of <100 ng/mL, measured on standardized clinical laboratory platform, seems to be useful for ruling out AKI in children with normal baseline renal function undergoing cardiac surgery [27, 28]. At 2 h after cardiac surgery, uNGAL at the 100-ng/mL cut-point had an AuROC of 0.95, sensitivity of 82%, and specificity of 90% for detection of a clinical diagnosis of AKI confirmed by an increase in serum creatinine. Overall, analytical concordance and agreement among the three assays were very good at this cutoff, with concordance values ranging from 97.3% to 99.1%. The optimal cut-point for NGAL in adult populations with baseline CKD risk factors or evident CKD is unknown [15, 27]. In a systematic review by the NGAL Meta-analysis Investigator Group, the best cutoff for AKI across all settings was 190.2 ng/mL, while for AKI prediction using uNGAL, it was 193.2 ng/mL [10]. We therefore evaluated concordance among the three assays at 190 ng/mL and observed similarly good concordance and agreement.

The classification of patients was very similar among the three assays: compared to Architect, there were 0–2 false positives and 1 false negative with the two BioPorto assays at a cutoff of 100 ng/mL. At the higher cutoff of 190 ng/mL, there were 0–1 false positives and

1 false negative with the two BioPorto assays compared to Architect. It is likely that serial monitoring of uNGAL and traditional biomarkers of creatinine and urine output would clarify the renal status of such misclassified patients, but testing of this hypothesis is beyond the scope of the current study. Although agreement was good at the two tested cutoffs, this will need to be further evaluated as data and recommendations regarding cutoffs for patients with CKD, sepsis, and other relevant confounders evolve.

Our study has some important strengths. To our knowledge, this is the first evaluation of the new nephelometric method for uNGAL determination. Also, we used specimens from actual patients in whom the test is likely to be used, thus representing uNGAL concentrations likely to be encountered in clinical practice. We acknowledge important limitations. Our sample size was modest and limited to the ICU, with a disproportionate number of septic ICU patients with stage 3 AKI. We therefore tended to have higher uNGAL levels in most of our patients. It would be important to verify our results in a broader patient population. We also did not normalize the uNGAL concentrations for urinary creatinine. However, it is debatable whether normalization for urine creatinine should be the standard. In animal as well as computer simulation studies, normalized levels of a biomarker reflecting tubular injury can be influenced by dynamic changes in the urinary creatinine excretion rate when the glomerular filtration rate changes. Normalization by urine creatinine concentration may therefore result in either an underestimation or an overestimation of the biomarker excretion rate depending on the clinical context [31, 32]. Despite these limitations, our study addresses relevant concerns regarding analytical harmonization of commercial clinical laboratory platforms for novel AKI biomarkers.

Conclusions

All three automated uNGAL methods showed acceptable analytical performance, and correlated well with each other. Although the three assays were broadly comparable, on the average, Architect yields lower results than both Dimension Vista and Advia 2400. This bias was more pronounced at higher uNGAL concentrations. There was good analytical concordance among the three assays at the two tested cutoffs of 100 and 190 ng/mL in the studied ICU population. It is important to validate these findings in a broader patient population. Since uNGAL concentrations are affected by underlying CKD and sepsis, additional standardization efforts will likely be necessary to better harmonize the uNGAL methods at various clinically relevant cutoffs.

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