

ORIGINAL INVESTIGATIONS

Pathogenesis and Treatment of Kidney Disease

Accuracy of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Diagnosis and Prognosis in Acute Kidney Injury: A Systematic Review and Meta-analysis

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) appears to be a promising biomarker for the early diagnosis of acute kidney injury (AKI); however, a wide range in its predictive value has been reported.

Study Design: Meta-analysis of diagnostic test studies using custom-made standardized data sheets sent to each author.

Setting & Population: Different clinical settings of AKI.

Selection Criteria for Studies: MEDLINE, EMBASE, and CENTRAL databases and congress abstracts were searched for studies reporting the value of NGAL to predict AKI.

Index Tests: Plasma/serum and urine NGAL within 6 hours from the time of insult (if known) or 24-48 hours before the diagnosis of AKI if the time of insult was not known.

Reference Tests: The primary outcome was AKI, defined as an increase in serum creatinine level > 50% from baseline within 7 days or contrast-induced nephropathy (creatinine increase > 25% or concentration > 0.5 mg/dL in adults or > 50% increase in children within 48 hours). Other outcomes predicted using NGAL were renal replacement therapy initiation and in-hospital mortality.

Results: Using a hierarchical bivariate generalized linear model to calculate the diagnostic odds ratio (DOR) and sample size-weighted area under the curve for the receiver-operating characteristic (AUC-ROC), we analyzed data from 19 studies and 8 countries involving 2,538 patients, of whom 487 (19.2%) developed AKI. Overall, the DOR/AUC-ROC of NGAL to predict AKI was 18.6 (95% CI, 9.0-38.1)/0.815 (95% CI, 0.732-0.892). The DOR/AUC-ROC when standardized platforms were used was 25.5 (95% CI, 8.9-72.8)/0.830 (95% CI, 0.741-0.918) with a cutoff value > 150 ng/mL for AKI compared with 16.7 (95% CI, 7.1-39.7)/0.732 (95% CI, 0.656-0.830) for "research-based" NGAL assays. In cardiac surgery patients, the DOR/AUC-ROC of NGAL was 13.1 (95% CI, 5.7-34.8)/0.775 (95% CI, 0.669-0.867); in critically ill patients, 10.0 (95% CI, 3.0-33.1)/0.728 (95% CI, 0.615-0.834); and after contrast infusion, 92.0 (95% CI, 10.7-794.1)/0.894 (95% CI, 0.826-0.950). The diagnostic accuracy of plasma/serum NGAL (17.9 [95% CI, 6.0-53.7]/0.775 [95% CI, 0.679-0.869]) was similar to that of urine NGAL (18.6 [95% CI, 7.2-48.4]/0.837 [95% CI, 0.762-0.906]). We identified age to be an effective modifier of NGAL value with better predictive ability in children (25.4 [95% CI, 8.9-72.2]/0.930 [95% CI, 0.883-0.968]) compared with adults (10.6 [95% CI, 4.8-23.4]/0.782 [95% CI, 0.689-0.872]). NGAL level was a useful prognostic tool with regard to the prediction of renal replacement therapy initiation (12.9 [95% CI, 4.9-33.9]/0.782 [95% CI, 0.648-0.917]) and in-hospital mortality (8.8 [95% CI, 1.9-40.8]/0.706 [95% CI, 0.530-0.747]).

Limitations: Serum creatinine level was used for AKI definition.

Conclusions: NGAL level appears to be of diagnostic and prognostic value for AKI.

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INDEX WORDS: Neutrophil gelatinase-associated lipocalin (NGAL); plasma NGAL; urine NGAL; meta-analysis; acute kidney injury (AKI).

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Acute kidney injury (AKI) is a frequent and

serious complication of hospitalized patients associated with substantial morbidity and mortality.¹⁻³ Despite significant advances in our understanding of the pathophysiologic characteristics of

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AKI, several interventions for AKI have been proved ineffective.⁴⁻⁶ This failure is caused in part by the lack of real-time sensitive and specific renal biomarkers to allow the early diagnosis of impending AKI. In current clinical practice, serum creatinine level and urine output are the most frequently used indicators of renal dysfunction despite their known limitations. They have limited sensitivity and specificity and creatinine level has a slow rate of change, thus limiting their usefulness in the early detection of AKI.⁷ Consensus groups, such as the Acute Dialysis Quality Initiative (ADQI), the AKI Network (AKIN), and the American Society of Nephrology, have set the development and validation of novel biomarkers of AKI as a priority.^{8,9} Early detection of AKI could permit the institution of timely renal salvage therapies, which, with associated monitoring of response to therapy, may result in preserved renal function and avoidance of renal replacement therapy (RRT) requirement and perhaps translate into improved patient morbidity and mortality.

Genomic, transcriptomic, and proteomic techniques have identified neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of AKI.^{10,11} In experimental and clinical studies, NGAL has been investigated extensively and would appear to be one of the most frequently investigated and most promising early biomarkers of AKI.¹²⁻³² NGAL has been investigated across a range of different clinical settings of AKI, such as after cardiac surgery,¹⁵⁻¹⁷ in critically ill patients,^{18,19} in patients receiving intravenous contrast media infusion for coronary angiography,^{20,21} and in patients admitted to the emergency department.¹⁴ It also has been studied in both adult^{15,17} and pediatric^{13,16,18,19} populations and has been measured in urine and plasma/serum using “research-based” assays (NGAL Rapid enzyme-linked immunosorbent assay [ELISA] kit; Bioporto, Gentofte, Denmark)^{13,15} versus measurement using standardized clinical laboratory platforms (Triage kit [Biosite Inc, San Diego, CA] or ARCHITECT platform [Abbott Diagnostics, Abbott Park, IL]).^{16,17}

However, with accumulating evidence, conflicting observations raise concern about the robustness of NGAL as a biomarker. To address this issue, we performed a systematic review and meta-analysis of observational studies to estimate the diagnostic and prognostic accuracy of

NGAL and identify potential confounders or effective modifiers of its value in AKI. We hypothesized that NGAL level is of diagnostic and prognostic value, both overall and across a range of subgroups developing AKI; urine and plasma/serum NGAL levels are both valuable for the early diagnosis of AKI; NGAL level performs better in children; and there might be an advantage for standardized measurement of NGAL with clinical laboratory platforms.

METHODS

Data Sources and Search Strategy

The Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines for the conduct of meta-analyses of observational cohort studies³³ were followed. Two investigators (M.H. and P.D.) independently searched MEDLINE (through PubMed interface), EMBASE, CENTRAL, and Congress abstracts (to May 31, 2009) to identify potentially relevant articles or abstracts. Our search included 1 search term: neutrophil gelatinase-associated lipocalin or NGAL. There were no language restrictions. We reviewed the bibliographies of all selected articles to identify additional relevant studies.

Study Selection

Two reviewers (M.H. and P.D.) independently screened studies identified for inclusion and determined study eligibility. Disagreements were resolved by a third opinion (R.B.). Selection was restricted to published prospective cohort studies of humans investigating the diagnostic or prognostic accuracy of NGAL level to predict AKI, initiation of RRT, and in-hospital mortality. Trials enrolling patients in interventional studies and those exclusively with AKI were excluded.

Data Extraction and End Points

Most observational studies published for NGAL used different AKI definitions and timing of NGAL measurement, rendering data synthesis difficult, if not impossible. A custom-made standardized data collection sheet (Item S1; provided as supplementary online material available with this article at www.ajkd.org) was developed a priori (R.B., M.H., P.D., A.H.-F., and S.M.B.) for uniform definition of the primary study end point (prediction of AKI) and timing of NGAL measurement in relation to the diagnosis of AKI. This data sheet was sent by e-mail to each author of the studies selected and included the request for documentation and recalculation of the following variables: setting of AKI (patients after cardiac surgery, patients after application of contrast agents for coronary angiography, patients admitted to the intensive care unit, and patients admitted to the emergency department), sample size, age, sex, baseline serum creatinine level, number of patients with chronic kidney disease (estimated glomerular filtration rate < 60 mL/min using the 4-variable Modification of Diet in Renal

Disease [MDRD] Study equation³⁴), number of patients developing AKI (primary end point), those receiving RRT, and those who died in-hospital (secondary end points). AKI was defined according to the creatinine criteria of the RIFLE classification³⁵ (increase in serum creatinine > 50% from baseline within 7 days) or as contrast-induced nephropathy (increase in serum creatinine > 25% or 0.5 mg/dL in adults³⁶ or > 50% in children within 48 hours).²³ Such a time limit for the RIFLE classification was not officially set, but was implied by the general view of the ADQI Consensus Conference Group that AKI should occur acutely over a few days to a week. In addition, the data sheet requested information about the biological material NGAL was measured in (urine and plasma/serum) and the measurement method (research-based assays vs standardized clinical laboratory platforms) and antibodies used. The authors of each study determined the paired sensitivity and specificity (95% confidence interval [CI]) for the cutoff value of NGAL calculated to be closest to the left upper corner of the receiver operating characteristic (ROC) space to predict AKI, RRT initiation, or in-hospital mortality. The timing of NGAL measurement in relation to the development of AKI was considered. When the time of renal insult was known, NGAL measurement within 6 hours was used, and when the time of renal insult was unknown, measurement 24-48 hours before the diagnosis of AKI was used for calculation of performance characteristics. In addition, calculation of the area under the curve for the ROC (AUC-ROC) with 95% CIs was requested for the primary and secondary end points. Data about the quality of NGAL sample collection, processing, and storage also were recorded.

Of 26 requests for completion of the data collection sheet sent to authors, 19 completed forms with 23 data sets (4 studies measured NGAL in both plasma/serum and urine;

Table 1) were returned for data extraction. Two reviewers (M.H. and A.H.-F.) independently extracted data from data collection sheets.

Statistical Analysis

The publication bias of included studies was assessed using effective sample-size funnel plot (diagnostic odds ratio [DOR] values vs sample size of each study).³⁷ The primary statistical analysis was based on a hierarchical bivariate generalized linear mixed model as proposed by Harbord et al³⁸ using the Stata program METANDI (Stata-Corp LP, College Station, TX) by Harbord. Based on this model, the elliptical joint confidence region for sensitivity and specificity and a summary ROC curve were constructed and the DOR and 95% CI were determined. In addition, AUC-ROC values with 95% CI and cutoff values for NGAL weighted for sample size were pooled.³⁹⁻⁴¹ An AUC-ROC > 0.70 defines a useful risk predictor.⁴² The I^2 statistic was calculated to determine the proportion of between-study variation in the AUC-ROC caused by heterogeneity, with suggested thresholds for low (25%-49%), moderate (50%-74%), and high (>75%) values.⁴³

RESULTS

Search Results and Study Characteristics

The electronic database searches identified 244 citations of studies and abstracts. After evaluating these citations and the bibliographies of included studies, we included 19 studies with 23 data sets.^{13-20,22-32} These comprised data from 4

Table 1. Characteristics of Studies

Reference	Sample Size	Population Type	Age (y)	Women (%)	Mean Baseline Serum Creatinine (mg/dL)	Impaired Renal Function (%)	Setting	NGAL Measurement	Country
Mishra et al, 2005 ¹³	71	Children	3.0	36.6	0.45	0	CS	Plasma + urine	United States
Wagener et al, 2006 ¹⁵	81	Adults	64.7	34.6	1.10	32.1	CS	Urine	United States
Dent et al, 2007 ²⁵	123	Children	4.2	48.8	0.50	0	CS	Plasma	United States
Zappitelli et al, 2007 ¹⁸	39	Children	7.1	48.7	0.44	0	ICU	Urine	United States
Hirsch et al, 2007 ²³	91	Children	6.9	44.0	0.73	0	CIN	Plasma + urine	United States
Wagener et al, 2008 ²⁶	426	Adults	63.2	33.8	1.08	27.2	CS	Urine	United States
Bennett et al, 2008 ¹⁶	196	Children	4.0	46.4	0.39	0	CS	Urine	United States
Ling et al, 2008 ²⁰	40	Adults	67.9	40.0	0.83	0	CIN	Urine	China
Koyner et al, 2008 ²²	72	Adults	61.3	29.2	1.24	26.4	CS	Plasma + urine	United States
Nickolas et al, 2008 ¹⁴	541	Adults	59.2	48.4	1.20	26.8	ED	Urine	United States
Lima et al, 2008 ²⁷	52	Adults	54.7	42.3	1.20	53.8	CS	Urine	Brazil
Wheeler et al, 2008 ¹⁹	143	Children	2.2	28.0	0.76	—	ICU	Plasma	United States
Xin et al, 2008 ²⁸	33	Children + adults	38.0	42.4	0.77	0	CS	Urine	China
Cruz et al, 2009 ²⁹	301	Adults	58.6	31.2	0.97	6.7	ICU	Plasma	Italy
Makris et al, 2009 (CIN) ³⁰	60	Adults	62.8	18.3	0.86	13.3	CIN	Urine	Greece
Makris et al, 2009 (ICU) ³¹	31	Adults	41.9	19.4	0.97	—	ICU	Urine	Greece
Tuladhar et al, 2009 ²⁴	50	Adults	66.7	30.0	1.10	42.0	CS	Plasma + urine	United Kingdom
Constantin et al, 2009 ³²	88	Adults	57.0	45.5	0.81	—	ICU	Plasma	France
Haase-Fielitz et al, 2009 ¹⁷	100	Adults	69.5	39.0	1.04	27.0	CS	Plasma	Australia

Note: Conversion factor for serum creatinine in mg/dL to $\mu\text{mol/L}$, $\times 88.4$.

Abbreviations and definitions: CIN, contrast-induced nephropathy; CS, cardiac surgery-associated acute kidney injury; ED, emergency department; ICU, intensive care unit; NGAL, neutrophil gelatinase-associated lipocalin.

conference abstracts.^{27,29,30,32} A flow chart detailing the process of study identification and selection is shown in Fig 1, and characteristics of included studies are listed in Table 1. The Cohen κ statistic for agreement on study inclusion was 0.94. Three authors of 3 additional studies published to date in only abstract form (without AUC-ROC values, sensitivity, or specificity) decided not to provide the data requested because of possible impediment of their original publication in preparation. One research group responsible for 4 publications unfortunately was not able to calculate the AUC-ROC because of the lack of an appropriate statistical software program.

Although all studies were published in English, they represent an international experience, having been conducted in 8 countries. All studies were single-center trials and excluded patients on long-term hemodialysis therapy before enrollment in the study. A total of 2,538 patients (mean sample size, 123 [95% CI, 67-179]; median, 72 [25th-75th percentiles, 50-123]) was enrolled in these studies. Study populations included adults and children studied in different AKI settings. Studies most frequently investigated AKI after cardiac surgery, followed by AKI in critically ill patients and after exposure to contrast media for coronary angiography. More studies measured NGAL in urine compared with plasma/serum and in adults compared with children. NGAL measurements most commonly were obtained using research-based assays.^{13-15,18-20,22-24,26-28,30,31} However, some recent studies have used standardized clinical laboratory platforms with a chemilumines-

cent microparticle assay, such as the Triage kit, for plasma NGAL^{17,25,29,32} and the ARCHITECT platform for urine NGAL.¹⁶

Quality Assessment

All studies originally were designed to examine the diagnostic accuracy of NGAL level, enrolled consecutive patients prospectively, and had clearly defined eligibility criteria and reasons for patient exclusion (Table 2). For primary and secondary analyses of the diagnostic accuracy of NGAL, 2 studies^{15,17} provided sample-size estimation and the remaining studies used a convenience sample size. However, for the prognostic accuracy of NGAL level, all studies used a convenience sample size. In all studies, the spectrum of patients was representative of patients who might receive the test in practice. Serum creatinine was used and measured using a modified Jaffé method. No patient underwent renal scintigraphy for verification of AKI diagnosis. Several studies reported investigators' involvement with industry sponsors. In no case was data extraction, statistical analysis, or drafting of the manuscript influenced by the sponsors. Laboratory personnel measuring serum creatinine in the hospital pathology department and clinicians were not aware of the NGAL source and results, which was measured by spatially divided "research" personnel. Research personnel were blinded to sample sources, clinical outcomes, and serum creatinine concentrations. All studies had similar sample collection and processing. Most stored samples at -80°C ; however, 2 studies stored samples at -20°C , 1 of those for short term³¹ and 1 for long term.²⁶

There was no evidence of significant publication bias for the primary or secondary outcomes (regression test of asymmetry, $P > 0.2$). Three studies directly compared the performance of NGAL level with that of serum creatinine level.^{14,17,31} For NGAL and serum creatinine levels, Nickolas et al¹⁴ reported similar AUC-ROC (0.95 vs 0.92) values for the early diagnosis of AKI defined as a new-onset 1.5-fold increase in serum creatinine level or a 25% decrease in estimated glomerular filtration rate from baseline in a cohort of patients admitted to the emergency department. Makris et al³¹ and Haase-Fielitz et al¹⁷ found a significantly greater AUC-ROC value for NGAL level at admission to the intensive care

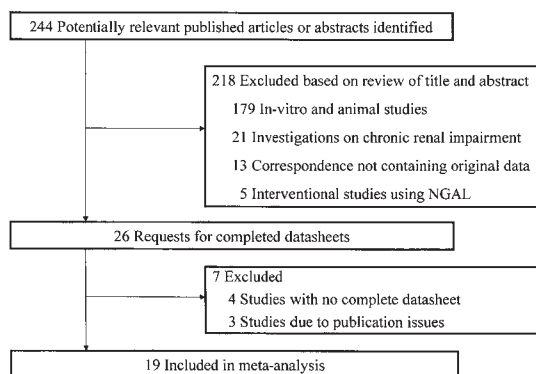


Figure 1. Flow of study selection. Abbreviation: NGAL, neutrophil gelatinase-associated lipocalin.

Table 2. Design Characteristics of Individual Studies

Reference	Design	Eligibility Criteria Clearly Defined	Sample Size Estimation	Representativeness of Patients (within setting)	Blinding	Funding	Storage	NGAL Assay
Mishra et al, 2005 ¹³	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Wagener et al, 2006 ¹⁵	P, C	Yes	Yes	Yes	Yes	No	< -70°C	Immunoblot ^{a,b}
Dent et al, 2007 ²⁵	P, C	Yes	No	Yes	Yes	SM-B	< -70°C	ELISA ^{d,e}
Zappitelli et al, 2007 ¹⁸	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Hirsch et al, 2007 ²³	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Wagener et al, 2008 ²⁶	P, C	Yes	No	Yes	Yes	No	-20°C	ELISA ^{a,b}
Bennett et al, 2008 ¹⁶	P, C	Yes	No	Yes	Yes	SM-A	< -70°C	ELISA ^{d,e}
Ling et al, 2008 ²⁰	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,c}
Koyner et al, 2008 ²²	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Nickolas et al, 2008 ¹⁴	P, C	Yes	No	Yes	Yes	No	< -70°C	Immunoblot ^{a,b}
Lima et al, 2008 ²⁷	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Wheeler et al, 2008 ¹⁹	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Xin et al, 2008 ²⁸	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Cruz et al, 2009 ²⁹	P, C	Yes	No	Yes	Yes	SM-B	< -70°C	ELISA ^{d,e}
Makris et al, 2009 (CIN) ³⁰	P, C	Yes	No	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Makris et al, 2009 (ICU) ³¹	P, C	Yes	No	Yes	Yes	No	-20°C	ELISA ^{a,b}
Tuladhar et al, 2009 ²⁴	P, C	Yes	Yes	Yes	Yes	No	< -70°C	ELISA ^{a,b}
Constantin et al, 2009 ³²	P, C	Yes	No	Yes	Yes	SM-B	< -70°C	ELISA ^{d,e}
Haase-Fielitz et al, 2009 ¹⁷	P, C	Yes	Yes	Yes	Yes	SM-B	< -70°C	ELISA ^{d,e}

Note: For patients admitted to the emergency department (excluding prerenal azotemia, n = 541).¹⁴

Abbreviations and definitions: CIN, contrast-induced nephropathy; ELISA, enzyme-linked immunosorbent assay; ICU, intensive care unit; NGAL, neutrophil gelatinase-associated lipocalin; P, C, prospective cohort study; SM-A, sample measurement funded by Abbott; SM-B, sample measurement funded by Biosite.

^aResearch-based assay.

^bBioporto, Gentofte, Denmark.

^cR & D Systems, Minneapolis, MN.

^dStandardized clinical laboratory platforms (Triage kit [Biosite, San Diego, CA] or ARCHITECT platform [Abbott, Abbott Park, IL]).

^eNGAL measurement funded by Biosite or Abbott.

unit (0.98 vs 0.79 and 0.80 vs 0.68, respectively) predicting subsequent AKI compared with serum creatinine level.

Evidence Synthesis

In Table 3, true-positive, false-positive, false-negative, true-negative, paired sensitivity and specificity, and cutoff values of individual studies are listed for NGAL level to predict AKI. In Table 4, the same indices for NGAL level to predict RRT or in-hospital mortality are listed.

In Table 5, the diagnostic and prognostic accuracy of NGAL weighted for sample size is shown. Across settings of AKI,^{13-20,22-32} we found a DOR of 18.6 for NGAL at sensitivity of 76.4% and specificity of 85.1% (Fig 2) and AUC-ROC of 0.815.

Subset analyses showed some variability in DOR and AUC-ROC values. NGAL level was of

diagnostic value for AKI after cardiac surgery, in critically ill patients, after contrast media use, and in patients admitted to the emergency department. The best predictive performance of NGAL level was found in the setting of AKI after exposure to contrast agents^{20,23,30} (DOR, 92.0; AUC-ROC, 0.894). The specificity of NGAL level for prediction of AKI after exposure to contrast media was > 95%, whereas it was ~75% after cardiac surgery and in critically ill patients at sensitivity of ~70%-75% in all AKI settings.

The DOR and AUC-ROC of plasma/serum NGAL level^{13-19,22-25,29,32} (DOR, 17.9; AUC-ROC, 0.775) were similar to those of urine NGAL level^{13-15,16,18,20,22,24,26-28,30,31} (DOR, 18.6; AUC-ROC, 0.837) in the prediction of AKI, with a slightly higher cutoff value for urine NGAL (Table 5). However, the value of NGAL level to predict AKI in children^{13,16,18,20,23,25}

Table 3. Paired Sensitivity and Specificity of Individual Studies for NGAL to Predict AKI

Reference	No. of Patients				NGAL Cutoff (ng/mL)	Sensitivity (%; 95% confidence interval)	Specificity (%; 95% confidence interval)
	TP	FP	FN	TN			
Mishra et al, 2005 (p) ¹³	14	3	6	48	>25	70.0 (45.7-87.2)	94.1 (82.8-98.5)
Mishra et al, 2005 (u) ¹³	20	1	0	50	>50	100.0 (80.0-100.0)	98.0 (88.2-99.9)
Wagener et al, 2006 ¹⁵	11	23	5	42	>400	68.8 (41.5-87.9)	64.6 (51.7-75.8)
Dent et al, 2007 ²⁵	38	5	7	73	>150	84.4 (69.9-93.0)	93.6 (85.0-97.6)
Zappitelli et al, 2007 ¹⁸	12	7	4	16	>10	75.0 (47.4-91.7)	69.6 (47.0-85.9)
Hirsch et al, 2007 (p) ²³	8	1	3	79	>100	72.7 (39.3-92.7)	98.8 (92.3-99.9)
Hirsch et al, 2007 (u) ²³	8	0	3	80	>100	72.7 (39.3-92.7)	100.0 (94.3-100.0)
Wagener et al, 2008 ²⁶	44	172	24	186	>450	64.7 (52.1-75.6)	52.0 (46.7-57.2)
Bennett et al, 2008 ¹⁶	78	8	21	89	>150	78.8 (69.2-86.1)	91.8 (83.9-96.1)
Ling et al, 2008 ²⁰	10	8	3	19	—	76.9 (46.0-93.8)	70.4 (49.7-85.5)
Koyner et al, 2008 (p) ²²	8	13	10	41	>280	44.4 (22.4-68.7)	75.9 (62.1-86.1)
Koyner et al, 2008 (u) ²²	12	19	6	35	>550	66.7 (41.2-85.6)	64.8 (50.6-77.0)
Nickolas et al, 2008 ¹⁴	20	16	3	502	>80	87.0 (65.3-96.6)	96.9 (94.9-98.2)
Lima et al, 2008 ²⁷	5	12	1	34	—	83.3 (36.5-99.1)	73.9 (58.6-85.3)
Wheeler et al, 2008 ¹⁹	19	74	3	47	>140	86.4 (64.0-96.4)	38.8 (30.3-48.2)
Xin et al, 2008 ²⁸	2	8	1	22	>250	66.7 (12.5-98.2)	73.3 (53.8-87.0)
Cruz et al, 2009 ²⁹	47	46	17	191	>150	73.4 (60.7-83.3)	80.6 (74.9-85.3)
Makris et al, 2009 (CIN) ³⁰	9	6	1	44	>60	90.0 (54.1-99.5)	88.0 (75.0-95.0)
Makris et al, 2009 (ICU) ³¹	6	7	1	17	>190	85.7 (42.0-99.3)	70.8 (48.8-86.6)
Constantin et al, 2009 ³²	43	1	9	35	>155	82.7 (69.2-91.3)	97.2 (83.8-99.9)
Tuladhar et al, 2009 (p) ²⁴	7	13	2	28	>420	77.8 (40.2-96.1)	68.3 (51.8-81.4)
Tuladhar et al, 2009 (u) ²⁴	8	9	1	32	>390	88.9 (50.7-99.4)	78.1 (62.0-88.9)
Haase-Fielitz et al, 2009 ¹⁷	18	17	5	60	>150	78.3 (55.8-91.7)	77.9 (66.8-86.3)

Abbreviations: AKI, acute kidney injury; CIN, contrast-induced nephropathy; FN, false negative; FP, false positive; ICU, intensive care unit; NGAL, neutrophil gelatinase-associated lipocalin; p, plasma; TP, true positive; TN, true negative; u, urine.

(DOR, 25.4; AUC-ROC, 0.930) was substantially higher compared with that in adults^{14,15,17,20,22,24,26,29-32} (DOR, 10.6; AUC-ROC, 0.782; Table 5). The study by Xin et al²⁸ was excluded from this comparison because both children and adults were enrolled (Table 5).

Across all settings of AKI, we investigated the diagnostic accuracy of NGAL level for AKI when measured using standardized clinical platforms^{16,17,25,29,32} versus research-based assays.^{13-15,18-20,22-24,26-28,30,31} Performance characteristics of NGAL level from this analysis also are listed in Table 5. We found higher DOR and AUC-ROC values for standardized assays (DOR, 25.5; AUC-ROC, 0.830) compared with individually developed research-based assays (DOR, 16.7; AUC-ROC, 0.732).

The cutoff NGAL concentration for optimal sensitivity and specificity to predict AKI across all settings ranged from 100-270 ng/mL, with higher values for adults (170 ng/mL) compared with children (100-135 ng/mL; Table 5).

However, there was large agreement on a cutoff value > 150 ng/mL when NGAL was measured using standardized platforms, contrasting with large variability in cutoff values derived from research-based NGAL assays (Table 5).

Despite some deviation, there was broad agreement between values originally reported and those recalculated for the meta-analysis. Generally, NGAL level performance improved when the definition of AKI used in this meta-analysis was more severe compared with the original publication and vice versa, in accordance with a previous observation.⁴⁴

The incidence of RRT reported in the included studies was 4.3%, and in-hospital mortality, 5.4%. In Table 5, data for the prognostic accuracy of NGAL level are listed. NGAL level appears to be a useful prognostic tool with regard to the prediction of the initiation of RRT (DOR, 12.9; AUC-ROC, 0.782; Fig 3) and, with some limitation, in-hospital mortality (DOR, 8.8; AUC-ROC, 0.706; Fig 4).

Table 4. Paired Sensitivity and Specificity of Individual Studies for NGAL to Predict RRT Initiation or In-Hospital Mortality

	No. of Patients				NGAL Cutoff (ng/mL)	Sensitivity (%; 95% confidence interval)	Specificity (%; 95% confidence interval)
	TP	FP	FN	TN			
NGAL to predict RRT							
Wagener et al, 2006 ¹⁵	4	45	1	31	>470	80.0 (29.9-99.0)	40.8 (29.8-52.7)
Wagener et al, 2008 ²⁶	5	150	3	268	>680	62.5 (25.9-89.8)	64.1 (59.3-68.7)
Bennett et al, 2008 ¹⁶	3	15	1	177	>150	75.0 (21.9-98.7)	92.2 (87.2-95.4)
Koyner et al, 2008 (p) ²²	5	28	2	37	>480	71.4 (30.3-94.9)	56.9 (44.1-68.9)
Koyner et al, 2008 (u) ²²	4	9	3	56	>570	57.1 (20.3-88.2)	86.2 (74.8-93.1)
Nickolas et al, 2008 ¹⁴	8	16	4	513	>80	66.7 (35.4-88.7)	97.0 (95.0-98.2)
Wheeler et al, 2008 ¹⁹	19	74	3	47	>140	86.4 (64.0-96.4)	38.8 (30.2-48.2)
Cruz et al, 2009 ²⁹	13	99	2	187	>150	86.7 (58.4-97.7)	65.4 (59.5-70.8)
Constantin et al, 2009 ³²	6	23	1	58	>300	85.7 (42.0-99.3)	71.6 (60.3-80.8)
Haase-Fielitz et al, 2009 ¹⁷	3	0	1	96	>340	75.0 (21.9-98.7)	100.0 (95.2-100.0)
NGAL to predict mortality							
Wagener et al, 2006 ¹⁵	5	46	1	29	>470	83.3 (36.5-99.1)	38.7 (27.9-50.6)
Wagener et al, 2008 ²⁶	13	234	3	176	>190	81.3 (53.7-95.0)	42.9 (38.1-47.9)
Bennett et al, 2008 ¹⁶	3	12	0	181	>150	100.0 (30.9-100.0)	93.8 (89.1-96.6)
Koyner et al, 2008 (p) ²²	3	34	1	34	>480	75.0 (21.9-98.7)	50.0 (37.7-62.3)
Koyner et al, 2008 (u) ²²	3	0	1	68	>570	75.0 (21.9-98.7)	100.0 (93.3-100.0)
Nickolas et al, 2008 ¹⁴	3	16	4	518	>80	42.9 (11.8-79.8)	97.0 (95.1-98.2)
Cruz et al, 2009 ²⁹	31	81	21	168	>150	59.6 (45.1-72.7)	67.5 (61.2-73.2)

Abbreviations: FN, false negative; FP, false positive; NGAL, neutrophil gelatinase-associated lipocalin; p, plasma; TP, true positive; TN, true negative; RRT, renal replacement therapy; u, urine.

DISCUSSION

The need for a simple, accurate, and minimally invasive marker of AKI has been a limiting factor in clinical nephrology research and practice. Although serum creatinine level is the current standard as such a marker, its limitations are well known and include its dependence on age, sex, and muscle mass.^{7,17} More accurate methods, such as radiolabelled tracer clearances, are invasive, may involve radiation, and require several hours to perform. Of several recently characterized novel renal biomarkers,⁴⁵⁻⁴⁷ NGAL has received the most interest. This interest has increased with the advent of point-of-care or rapid central laboratory measurement techniques for the standardized measurement of NGAL in clinical settings. However, a wide range of predictive values of NGAL for AKI have been reported across observational cohort studies.^{13,22,44}

We performed a systematic review and meta-analysis to clarify the predictive value of NGAL for the early diagnosis of AKI, both overall and across a range of subgroups developing AKI. We also investigated the predictive value of plasma/serum NGAL with urine NGAL level, applied in children versus adults, and measured using re-

search-based versus standardized assays for the early diagnosis of AKI.

First, we found NGAL level to be a useful early predictor of AKI, both overall and across a range of clinical settings. Second, urine or plasma/serum NGAL levels performed similarly well. Third, the performance of NGAL level improved when standardized clinical laboratory platforms with a cutoff NGAL concentration > 150 ng/mL were used, in comparison to research-based assays. Finally, NGAL level had prognostic value for clinical outcomes, such as initiation of RRT and mortality.

In the literature, different definitions of AKI,^{1,35,48} various settings of AKI,^{14,16,21,26} and varying timings of NGAL measurement with regard to a renal insult have been used to assess the predictive value of NGAL level, thus creating effective modifiers of NGAL's usefulness as a biomarker.⁴⁴ Also, a clear cutoff NGAL concentration for the detection of AKI has not yet been reported. This is the first meta-analysis of the performance of NGAL as a predictor of AKI and represents an attempt to address these issues and provide guidance for its future use. Our meta-analysis provides a systematic overview summa-

Table 5. Pooled Diagnostic and Prognostic Accuracy of NGAL

Setting (no. of events/total patients; no. of studies [data sets])	Sensitivity ^a (95% CI)	Specificity ^a (95% CI)	DOR ^a (95% CI)	AUC-ROC ^a (95% CI)	I ² (%)	NGAL Cutoff ^a (ng/mL)
AKI across settings (487/2,538; 19 [23])	76.4 (70.4-81.6)	85.1 (76.6-90.9)	18.6 (9.0-38.1)	0.815 (0.732-0.892)	43.5	190.2 (122.8-257.2)
AKI after cardiac surgery (307/1,204; 10 [13])	75.5 (70.2-82.4)	75.1 (65.2-86.3)	13.1 (5.7-34.8)	0.775 (0.669-0.867)	27.8	273.6 (145.0-289.2)
AKI in critically ill patients (123/602; 5 [5])	76.4 (59.9-87.5)	75.5 (52.2-89.7)	10.0 (3.0-33.1)	0.728 (0.615-0.834)	17.5	155.0 (150.8-169.0)
AKI after contrast infusion (34/191; 3 [4])	77.8 (62.8-88.0)	96.3 (74.4-99.6)	92.0 (10.7-794.1)	0.894 (0.826-0.950)	3.2	100.0 (80.0-100.0)
AKI in children across settings (213/663; 6 [8])	77.6 (69.7-83.9)	88.0 (75.8-94.5)	25.4 (8.9-72.2)	0.930 (0.883-0.968)	3.5	135.0 (50.0-150.0)
AKI in adults across settings (271/1,842; 12 [14])	72.5 (62.9-80.4)	80.1 (71.2-86.2)	10.6 (4.8-23.4)	0.782 (0.689-0.872)	27.5	175.0 (150.0-271.5)
AKI prediction using plasma/serum NGAL (226/1,039; 9 [9])	73.4 (62.3-82.2)	86.6 (72.0-94.3)	17.9 (6.0-53.7)	0.775 (0.679-0.869)	20.2	179.2 (153.9-199.3)
AKI prediction using urine NGAL (319/1,783; 14 [14])	77.8 (70.9-83.5)	84.3 (72.8-91.3)	18.6 (7.2-48.4)	0.837 (0.762-0.906)	21.9	193.2 (123.7-405.7)
AKI prediction using research-based assays (242/1,730; 14 [18])	76.9 (69.4-83.1)	83.4 (72.0-90.8)	16.7 (7.1-39.7)	0.732 (0.656-0.830)	31.6	246.4 (88.5-277.2)
AKI prediction using standardized platforms (245/808; 5 [5])	75.4 (63.8-84.2)	89.3 (81.9-93.9)	25.5 (8.9-72.8)	0.830 (0.741-0.918)	7.0	150.6 (145.0-155.0)
Initiation of RRT across AKI settings (84/1,948; 9 [10])	76.0 (65.1-84.4)	80.3 (59.5-91.9)	12.9 (4.9-33.9)	0.782 (0.648-0.917)	9.5	278.3 (141.9-381.6)
In-hospital mortality across AKI settings (88/1,617; 6 [7])	65.0 (51.2-80.8)	82.6 (51.8-95.5)	8.8 (1.9-40.8)	0.706 (0.530-0.747)	10.3	212.0 (121.8-506.7)

Note: NGAL measured within 6 hours from the time of renal insult or 24 to 48 hours before the diagnosis of AKI.

Abbreviations: AKI, acute kidney injury; AUC-ROC, area under the curve for the receiver-operating characteristic; CI, confidence interval; DOR, diagnostic odds ratio; NGAL, neutrophil gelatinase-associated lipocalin; RRT, renal replacement therapy.

^aWeighted for study sample size and calculated for NGAL concentration to be closest to the left upper corner of the ROC space. AUC-ROC and NGAL cut-off values denote median (25th-75th percentiles). I² values reflect heterogeneity of AUC-ROC values. The study by Xin et al²⁸ was excluded from separate children or adult analysis because both children and adults were enrolled in this study. Analysis of plasma/serum NGAL and urine NGAL included 4 studies providing data on NGAL's performance measured in both biological materials, plasma/serum, and urine. For patients admitted to the emergency department (excluding prerenal azotemia, n = 541),¹⁴ the predictive performance of urine NGAL for AKI was AUC-ROC of 0.975 (95% CI, 0.957-0.993); sensitivity, 86%; and specificity, 97% at a concentration > 80 ng/mL.

rizing data from observational studies for the diagnostic and prognostic accuracy of NGAL level to predict AKI using a uniform definition of AKI and standardized timing of NGAL measurements in relation to the renal insult. This approach enables direct comparison of results of these studies. This meta-analysis found that overall, NGAL is a valuable renal biomarker in all settings of AKI investigated. Although useful in adults, NGAL level has lower predictive value with wider CIs for AKI prediction compared with children. These findings deserve further investigation focusing on the influence of comorbidities, source of NGAL, and pathophysiologic characteristics of AKI.

Against the background that a renal biomarker with optimal performance in blood or urine is needed and the uncertainty of whether NGAL level performs better in urine^{13-16,18,20,22-24,26-28,30,31} or plasma/serum,^{13,17,19,22-25,29,32} this meta-analysis adds important data to the literature. Both plasma/serum and urine NGAL levels appear to perform similarly well and provide a relevant advantage compared with serum creatinine.

The cutoff value for NGAL was > 150 ng/mL in studies using standardized clinical platforms, whereas higher variability for cutoff and lower specificity were found in research-based NGAL assays. To what extent this cutoff value prevails currently is unknown and needs further investiga-

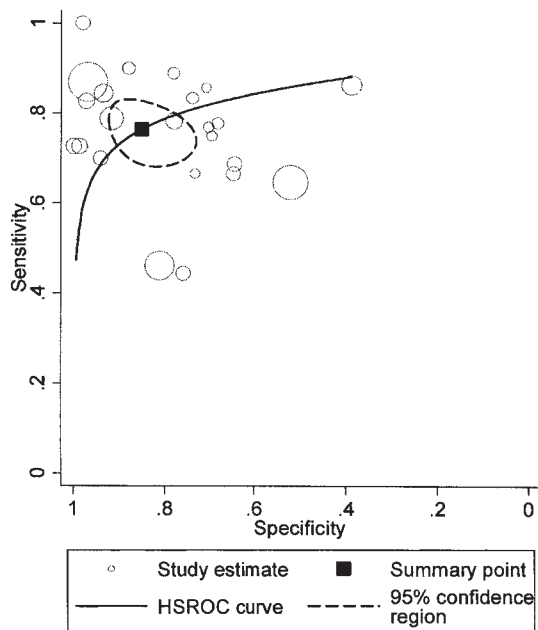


Figure 2. Hierarchical summary receiver operating characteristic (HSROC) plot of neutrophil gelatinase-associated lipocalin (NGAL) to predict acute kidney injury across settings. Based on combined sensitivity (95% confidence interval [CI]) and specificity (95% CI) weighted for sample size of each data set reflected by the size of the circles, showing average sensitivity and specificity estimate of the study results (solid square) and a 95% confidence region around it.

tion in large international prospective trials enrolling patients with normal or chronically impaired renal function in both patients developing AKI and those without AKI. However, it might be necessary for each center using NGAL level for early AKI diagnosis to define a specific normal range and cutoff value for each clinical setting.

The quality of data from completed data sheets was sufficient. Smaller studies often reported AUC-ROC values for AKI, but not for the initiation of RRT or in-hospital mortality, most likely because of the low incidence of these events. The effect of this data selection bias is unknown. However, for the primary outcome, completeness of data was achieved.

It is worth noting that in the studies included, the reference standard test for the diagnosis of AKI in current clinical practice was serum creatinine level despite its known limitations. None of the studies included used inulin or radiolabelled tracer-based clearance for more precise validation of NGAL's performance. Also, using NGAL

level as a single novel biomarker to predict AKI might not have covered all aspects of the diagnostic pathways for AKI, including kidney ultrasound and urine microscopy. However, NGAL level clearly predicted creatinine-based diagnosis of AKI and its associated morbidity and mortality. In this regard, we found NGAL level to be a useful marker in the early prediction of the initiation of RRT or in-hospital mortality, indicating that this renal biomarker has both diagnostic and prognostic relevance. NGAL was the only novel renal biomarker investigated, and we did not request data for the predictive performance of NGAL level normalized for urine creatinine concentration. However, this ratio was shown to increase transiently despite no change in the biomarker excretion rate simply because of the acute decrease in urine creatinine excretion after renal injury.⁴⁹ The influence of race and center-specific measures directed at the prevention of AKI on results of the meta-analysis is unknown.

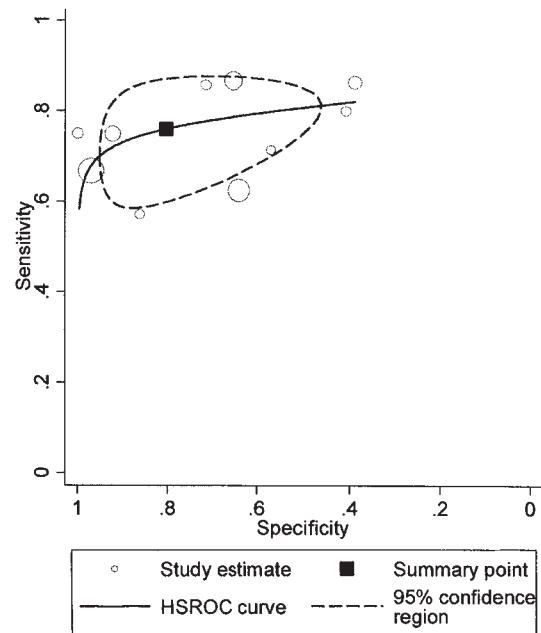


Figure 3. Hierarchical summary receiver operating characteristic (HSROC) plot of neutrophil gelatinase-associated lipocalin (NGAL) to predict the initiation of renal replacement therapy. Based on combined sensitivity (95% confidence interval [CI]) and specificity (95% CI) weighted for sample size of each data set reflected by the size of the circles, showing average sensitivity and specificity estimate of the study results (solid square) and a 95% confidence region around it.

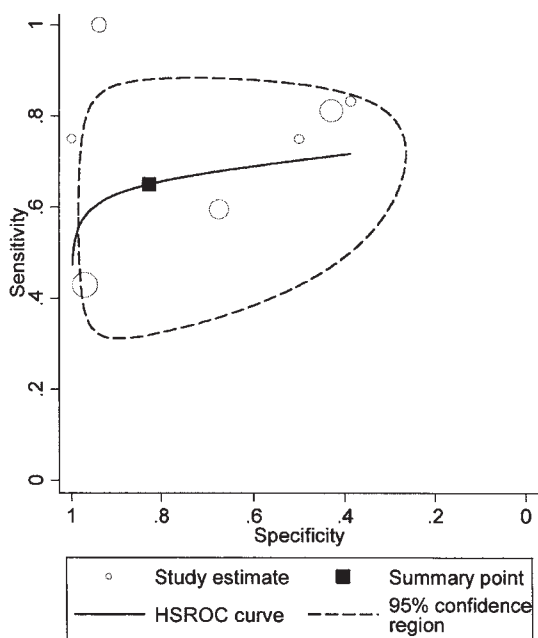


Figure 4. Hierarchical summary receiver operating characteristic (HSROC) plot of neutrophil gelatinase-associated lipocalin (NGAL) to predict in-hospital mortality. Based on combined sensitivity (95% confidence interval [CI]) and specificity (95% CI) weighted for sample size of each dataset reflected by size of circles, showing average sensitivity and specificity estimate of the study results (solid square) and a 95% confidence region around it.

Two of the 3 studies^{17,31} directly comparing the performance of NGAL level with that of serum creatinine level reported NGAL level to be superior to serum creatinine level for AKI prediction when measured at the same time.^{17,31} However, the study conducted by Nickolas et al¹⁴ did not find superiority of NGAL level over serum creatinine level predicting subsequent AKI in patients admitted to an emergency department given that patients already presenting with manifest AKI and those with subclinical AKI were pooled. One large study²⁶ with long-term storage of samples at -20°C showed a limited predictive value of NGAL level for AKI. Despite concerns of NGAL stability,⁵⁰ at this stage, there was no clear justification to exclude the study from this meta-analysis. Of enrolled patients, $\sim 70\%$ were from the United States.^{13-16,18,19,22,23,25,26} Although there were 2 studies from China^{20,28} and 1 from South America,²⁷ the generalizability of NGAL value needs further local investigation and validation. We did not use urine output for the definition of AKI because most studies could

not provide these data. However, the creatinine criteria of the RIFLE classification for the definition of AKI are considered to be valuable.⁵¹

A custom-made data collection sheet completed and returned by each author was used for all data analyzed. This implied recalculation of all performance characteristics of NGAL level for each study using a uniform definition of AKI and enabled a standardized data synthesis. Also, analysis of the performance characteristics of NGAL was limited to its measurement well in advance of the diagnosis of AKI, emphasizing its value as an early biomarker compared with serum creatinine level.

NGAL measurement is considerably less labor and time intensive than clearance techniques and will cause less patient inconvenience and morbidity. Given that NGAL level shows performance for AKI similar to that of troponin level for myocardial infarction during its clinical implementation period (AUC-ROC in the 0.7 range),⁵² NGAL appears to be a promising candidate as an enrollment criterion for clinical trials investigating early novel interventions or therapeutic drug monitoring/safety across settings of AKI.⁵³

It is possible that the diagnostic performance of NGAL will improve in the future as more studies are completed using standardized platforms rather than research-based assays (similar to what happened with troponin). More studies using standardized platforms with on-site measurement using fresh blood or urine samples from patients in multiple AKI settings may contribute to more robust laboratory implementation of NGAL.

In conclusion, in our meta-analysis of data from 19 studies including $> 2,500$ patients, NGAL level appears to be of diagnostic and prognostic value for AKI. When confirmed in large prospective studies, NGAL level should be considered in randomized controlled trials of preventive and therapeutic interventions in AKI.

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SUPPLEMENTARY MATERIAL

Item S1: Standardized data collection sheet.

Note: the supplementary material accompanying this article (doi:10.1053/j.ajkd.2009.07.020) is available at www.ajkd.org.

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