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Department of Surgery and Interventional Science, Royal Free Campus, University College London, 9th Floor Royal Free London NHS Foundation Trust, Pond Street, NW3 2QG.

Running Title: The role of NGALs post liver transplant.

Key Words: Liver Transplantation; Acute Kidney Injury (AKI); Biomarkers; Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Corresponding Author:

Francis P Robertson

Department of Surgery and Intervention Sciences, Royal Free Campus, University College London, 9th Floor, Royal Free Hospital, Pond Street, NW3 2QG.

Email: francis.robertson.13@ucl.ac.uk.

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Abstract

Background

Acute kidney injury (AKI) is common after orthotopic liver transplantation (OLT) usually occurring early post-transplant. Multiple causes include graft preservation injury, blood loss, hypotension but also severity of recipient liver disease. Early intervention in AKI has both short and long term patient benefits. Unfortunately there are no current clinical biomarkers of early AKI.

Aim

To assess the value of NGAL in predicting AKI following OLT.

Methods:

Ovid MEDLINE and EMBASE were searched between the years of 2000 and 2017 for studies using keywords : Neutrophil Gelatinase Associated Lipocalin or NGAL variants combined with synonyms for liver transplantation.

Results:

96 studies were identified. 11 studies including 563 patients were considered suitable for analysis. Both urinary (uNGAL) and plasma NGAL (pNGAL) measurement were found to predict AKI after liver transplantation. Optimal reported area under the receiver-operator characteristics curve (AUROC) values of 0.5-0.83 and 0.54-0.86 respectively.

Conclusions:

NGAL is a good predictor of early AKI post OLT although there is considerable variation in the published results. Further studies with prospectively defined cut-off values, standardized definitions of AKI and rigorous data reporting should be conducted to establish its clinical usefulness and limitations.

Introduction

Orthotopic Liver Transplantation (OLT) is the definitive intervention for patients with end-stage liver disease (ESLD). Acute kidney injury (AKI) is a common and serious complication occurring after liver transplantation. The published incidence ranges from 14% - 97% [1-4], with the variation partly arising from differences in diagnostic criteria. The development of AKI after liver transplant in the short term leads to prolonged hospital length of stay [1] and is associated with reduced patient and graft survival [2]. In the longer-term AKI leads to higher rates of Chronic Kidney Disease (CKD) and the need for long term dialysis or renal transplant [3, 4].

Diagnosis of AKI

The current gold standard for the diagnosis and classification of AKI is the measurement of change in serum creatinine (sCr) graded according to the RIFLE [5] (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) and AKIN [4] (Acute Kidney Injury Network) classifications.

SCr can vary widely with differences in age, muscle mass, hydration status, and often does not rise until significant kidney function is lost[6]. Furthermore creatinine levels are altered by liver function, the volume of ascites and bilirubin levels, which are frequently altered in the post-operative period of liver transplantation, and therefore are especially unreliable in this population[7].

In Acute Kidney injury an initial insult sets off an inflammatory cascade leading to cell death and extension of the initial injury. Hence the early phase of injury is the most promising for an intervention aimed to stop progression to established AKI [8]. The use of novel biomarkers could allow earlier identification of tubular injury and early initiation of intervention[9].

Neutrophil Gelatinase-Associated Lipocalin

Neutrophil Gelatinase-Associated Lipocalin (NGAL) has shown promise as a marker of acute kidney injury. NGAL is a 25kDA protein with key functions in innate immunity, iron binding, epithelial differentiation and the oxidative stress response [10]. Gene expression studies found NGAL to be highly upregulated in AKI [11] .

In animal models of acute kidney injury NGAL is detected in both urine and plasma within hours of insult [12-15]. Clinical trials have shown it to be an early marker of AKI peri-operatively in cardiac surgery [16, 17], kidney transplantation [18] and many other settings [19]. Higher NGAL values are predictive of more severe AKI as well as being an independent predictor of patient mortality and loss of renal function [20, 21].

NGAL for AKI in Liver Transplantation

Liver transplant patients are usually in advanced stages of liver disease at the time of operation. NGAL is upregulated within the liver in acute-on-chronic liver injury [22]. This could affect the NGAL levels in patients with hepatic impairment undergoing liver transplantation. NGAL has, however, been shown to differentiate AKI from

pre-renal azotaemia and hepato-renal syndrome in cirrhotic patients [23]. While NGAL has shown promise in a variety of settings it remains important to validate this marker independently in liver transplant surgery patients.

Methods

Search Strategy

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The study protocol was registered with the University of York Centre for Reviews and Dissemination international prospective register of systematic reviews (CRD42016037115).
The medical literature was searched for human studies on the diagnostic accuracy of urinary and plasma NGAL levels for the detection of AKI following liver transplantation. All data from papers and conference abstracts were included in the analysis. Only English language papers were included. Unpublished data was not sought.

MEDLINE and EMBASE databases were searched for the period 1st of January 2003 to 7th May 2017. The following search algorithm was used: (neutrophil gelatinase associated lipocalin OR NGAL$).mp. AND (Liver Transplantation/ OR ((liver or hepatic) adj3 (transplant$ or graft$00.mp.) Two authors independently reviewed the titles and abstracts of relevant studies identified during the search.

Study Selection

We included all prospective cohort studies that examined the diagnostic effect of urinary or plasma NGAL in the setting of AKI after human liver transplantation. We excluded all animal studies, editorial or review papers, and studies that examined

chronic renal failure, other forms of hepatobiliary surgery, involved other causes of AKI or its prevention following liver transplantation (e.g. tacrolimus induced injury, sevoflurane induced protection). We included all adult patients (aged 18 and over) who underwent urgent or elective liver only transplant for End Stage Liver Disease. There were insufficient data for a meaningful data synthesis and meta-analysis and therefore a systematic review only was undertaken.

Two independent authors reviewed the full published articles using the validated Quality Assessment of Diagnostic Accuracy Studies version 2 (QUADAS-2) tool [24]. Disagreements were resolved by consensus or where necessary by the senior author.

Results

The initial search identified 96 titles (Figure 1), of which 72 were excluded as duplicates or based on content of title or abstract. 24 full papers were reviewed of which 13 were excluded. Of the 13 exclusions 4 studied chronic renal failure, 4 were editorials or review papers and 4 studies involved specific causes of AKI. A single publication presented data that was not extractable for the purpose of this study.

Eleven studies met all inclusion criteria, reporting on a total of 596 patients from 13 centres. Characteristics of the trials are shown in Table 1 and assessment using the QUADAS-2 [24] tool in Table 2.

Of 563 patients 52 (9.1%) received a Living Related Donor transplant. Twenty-two patients received Domino Transplants from Familial Amyloid Polyneuropathy (FAP) patient donors. Other studies either did not comment on the donor type or specified that all organs were whole organs from deceased donors.

Impaired baseline renal function was an exclusion criterion in most studies. Three studies [25-27] excluded any patient on renal replacement therapy or in end stage renal failure. Two studies [28, 29] excluded patients with any CKD history, but did not give specific criteria. One study [30] excluded any patient with diabetes or any history putting them at risk of AKI or CKD. One study [31] excluded patients with baseline creatinine <1.5mg/dL whilst another [32] only analysed those with baseline creatinine <1.5mg/dL. Khosravi *et al.* [33] excluded patients with baseline creatinine >2mg/dL, and further excluded any patients on cyclosporin or sirolimus immunosuppression, or with inflammatory renal conditions, anaemia, malignancy or hypertension. Marcelino *et al.* [34] excluded all patients with creatinine clearance of less than 60mL/min. Lewandowska *et al.* [35] had no renal function exclusion criteria and included 10 patients with renal impairment in their study.

Incidence of AKI in OLT and outcome

Five studies used the AKIN criteria [25, 28, 29, 32, 34] and five used RIFLE criteria [26, 27, 30, 31, 35] to diagnose AKI. Khosravi *et al.* [33] used only the 0.3mg/dL absolute increase in creatinine criterion of the AKIN classification. Sirota *et al.* [26] classified a creatinine rise diagnostic of AKI which resolved in 24 hours as pre-renal azotemia and did not include these patients in the AKI group, and this group also

reported the lowest rate of AKI (17.5%). A total of 227 of 563 patients (40%) developed AKI following OLT. Individual incidences of AKI after OLT for the 11 studies ranged from 17.5% [26] to 57.9% [30].

The studies were not powered or designed to evaluate the outcomes of patients developing AKI. Four studies did not report on outcomes of AKI [25, 26, 30, 33]. Two of the studies reported no significant differences in outcomes between their AKI and non-AKI groups [27, 29]. Lewandowska et al. [35] found the 28 day and 1 year mortality was significantly higher in the AKI when compared to the non-AKI group (15.5% and 25.9% vs. 0% and 3.9%, respectively, p <0.5). Marcelino et al. [34] found a significantly longer hospital stay following AKI (41.7 +/- 28.5 days in AKI group vs 31.3 +/-34.8 days in non-AKI group, p 0.01) Portal *et al.* [32] reported a median of 6 (range 4-12) days post-operative ITU stay for the AKI group in comparison to a median of 3 (3-4) days in non-AKI group (p<0.001).

Plasma NGAL (pNGAL)

Of the six studies [25, 28, 30-33] reporting on pNGAL for diagnosis of AKI post OLT, four [28, 30-32] reported significantly higher values in AKI than non-AKI patients. The AUROC of pNGAL for AKI ranged from 0.68 to 0.86 in these four studies. Details are presented in Table 3. Because of differences in methodology of the studies they are described individually.

Niemann *et al.* [31] found that the pNGAL levels at 2 hours post reperfusion of the liver graft were significantly higher in those who developed AKI [AUROC 0.79,

pNGAL 117 ± 30 ng/mL in non-AKI vs 156 ± 38ng/mL in AKI, p<0.05]. A cut-off value of 139ng/mL provided a sensitivity of 0.67 with specificity of 0.8. Dedeoglu *et al.* [28] analysed pNGAL at 4 and 8 hours following admission to ITU again with significant increases in pNGAL in those subsequently shown to develop AKI [AUROC 0.84, pNGAL 183ng/mL(100-268) in non-AKI vs 305ng/mL(253-523) in AKI group, p=0.004] and 8 hours [AUROC 0.86, pNGAL 180ng/mL(134-232) non-AKI vs 266ng/mL(249-391) in AKI, p=0.003]. Portal *et al.* [32] analysed pNGAL levels within 12 hours of ITU admission and found an AUROC of 0.79(0.68-0.87), p<0.001 for predicting AKI [127ng/mL(98-188) in non-AKI vs 157ng/mL(89-307) in the stage 1 AKI group], with an optimal cut-off of 212ng/mL giving sensitivity of 0.67 and specificity of 0.84. Plasma NGALs were accurate in differentiating mild from severe AKI (AKIN Stage 2 and above) with an AUROC of 0.87(0.77-0.93), p<0.001.

Jeong *et al.* [30] used a different approach by measuring serial pNGAL values and using an NGAL change of 50% from baseline as the cut-off for the diagnosis of AKI. Using this cut-off in 19 living related liver donor transplant recipients NGAL was significantly higher in AKI than non-AKI group at 2 hours post-reperfusion with AUROC 0.682(0.415-0.949), p=0.186 and peak values at 4 hours post-reperfusion.

Khosravi *et al*. [33] did not find pNGAL values at any individual time period to be significantly different between the AKI and non-AKI groups in 90 patients who underwent OLT. They reported a non-significant AUROC of 0.54 [pNGAL in AKI 193(+/-132.7)ng/mL vs 238.4(+/- 153.7)ng/mL, p=0.508]. However, by calculating

change in NGAL (ΔNGAL) at 2h post reperfusion from baseline, the study was able to determine AKI from non-AKI groups with AUROC of 0.64(0.52-0.76), p=0.029.

Cheng *et al.* [25] found pNGALs to peak at 2 hours post graft reperfusion in 22 patients. They reported on the diagnostic accuracy of the 1 hour pNGAL value and did not find this value alone to be significantly different between the groups. By determining the ratio between pNGAL concentration at 1h post reperfusion with pNGAL at anaesthetic induction, this group was able to predict AKI developing within 48hrs with good accuracy (AUROC 0.781, p=0.048).

Urinary NGAL measurement (uNGAL)

Three studies reported on uNGAL [26, 28, 32, 34] and four reported on uNGAL corrected for urinary creatinine (uNGAL/uCr). One study reported on both [34]. Of the 4 studies measuring absolute uNGAL values for the diagnosis of AKI, three found that uNGAL levels were significantly higher in those who were subsequently shown to develop AKI [26, 28, 32], with AUROCs ranging from 0.76 to 0.83. These studies did not report on sensitivity and specificity (Table 4). Marcelino *et al.* [34] reported no significant difference in absolute NGAL values between the AKI and non-AKI groups.

uNGAL/uCr Ratio

Correction of uNGAL value by urinary creatinine has been used to adjust for possible biomarker dilution caused by changes in urine output. Five studies used uCr

correction [27, 29, 30, 34, 35], with 4 using a retrospectively set cut-off [27, 29, 30, 34, 35]. Jeong *et al.* [30] used 50% change from baseline as the cut-off value. uNGAL to urinary Creatinine ratio (uNGAL/uCr) was predictive of AKI in four of the five studies which reported on this marker [27, 29, 30, 35] with AUROC ranging 0.65 to 0.8 in these studies. All studies measuring multiple timepoints reported peak values at 2-4h following reperfusion (Table 5).

Discussion

AKI is common post liver transplant and is associated with significant morbidity and mortality . AKI quickly leads to irreversible kidney damage and therefore the window for effective intervention is short. The recent ELAIN randomized trial investigated the effect of early versus delayed initiation of renal replacement therapy (RRT) in critically ill patients with AKI. Early RRT significantly reduced hospital stay (51 vs 82 days), 90 day mortality (39% vs 55%), and led to earlier recovery of renal function (90 day recovery 54% vs 39%) [36]. The use of novel biomarkers could allow earlier identification of tubular injury and new approaches to prevent AKI.

Furthermore, NGAL has been successfully used to differentiate transient creatinine rise from AKI in surgical patients [37]. In a meta-analysis of post-cardiac surgery and critically ill adults and children, NGAL detected subclinical kidney injury. Patients with raised NGAL and normal creatinine levels had increased rates of in-hospital mortality, prolonged critical care admission and increased need for renal replacement, when compared to patients with normal NGAL levels [38], suggesting NGALs can demonstrate occult renal injury.

Limited data is available on the use of NGAL for the identification of renal injury following liver transplantation. A systematic review was carried out of NGAL as an early biomarker for AKI following OLT to guide its future clinical use.

All three examined measures of NGAL – plasma, urine and urine corrected by urinary creatinine showed significantly higher NGAL levels in AKI patients following liver transplantation in most of the examined studies. The ranges for the best reported AUROCs were 0.54-0.86 for pNGAL [25, 28, 30-33], 0.5-0.83 for uNGAL [26-28, 32, 34] and 0.65-0.8 for uNGAL/uCr [27, 29, 30, 35].

pNGAL vs uNGAL

Three studies compared uNGAL and pNGAL directly with similar predictive values in head-to-head comparison [28, 30, 32]. Dedeoglu *et al.* [28] found uNGAL levels peaked and distinguished AKI and non-AKI hours earlier than pNGAL, but found similar predictive values at the respective optimal time-points.

Jeong *et al.* [30] measured pNGAL and uNGAL in 19 living-related donor liver transplant recipients.. In serial samples uNGAL/uCr was reported to peak at 2 hours post reperfusion while pNGAL peaked at 10 hours. Although the AUROC values were similar, the pNGAL measured may not have been at the optimal time point.

Portal *et al.* [32] found low urine output prevented uNGAL measurement in over half the patients. However, as oliguria is an independent clinical marker of AKI, this may not cause a significant limitation in clinical practice. Further direct comparison of

uNGAL and pNGAL with prospective cut-off points, and an analysis accounting for limitation in data collection, is necessary to establish which is more effective for use in routine clinical practice.

uCr correction

Urinary biomarker concentrations will be influenced by urine volume. AKI may or may not affect urine volume. As urinary creatinine concentration is also affected by urine volume, the ratio to uCr has been proposed as a method of correction for other urinary biomarkers. Postoperative uNGAL/uCr ratio was examined in 5 studies[27, 30, 34, 35]. uNGAL corrected for urinary creatinine did not outperform absolute uNGAL or pNGAL for the prediction of AKI. As yet, no consensus has been reached on whether uNGAL should be corrected by urine creatinine concentration. uNGAL linearly correlates with uCr, however limitations with large day-to-day variation and significant over-correction in uNGAL/uCr have been shown [39].

Sample Timing

In the studies examined NGAL measurements were timed from reperfusion [25, 29-31, 33, 34], end of surgery [26, 35] or ITU admission [28, 32]. Time of reperfusion is documented routinely in liver transplantation and is likely to best approximate onset of post-reperfusion syndrome, a major cause of AKI in OLT [40].

On serial measurement uNGAL/uCr peaked at 2 to 4 hours after reperfusion [27, 29, 30], and uNGAL at 4 hours from ITU admission[28], with coinciding peak AUROC

values. For pNGAL best discriminating ability for AKI (AUROC = 0.86) was reported by Dedeoglu *et al.* [28] at 8 hours after ITU Admission. Serial measurement by Jeong *et al.* [30] showed peak pNGAL values at 10 hours after reperfusion. Measurement of pNGAL within 2 hours post reperfusion required adjustment for baseline to show significant results in two studies [25, 33], but showed a good predictive value in a third [31]. No studies found 24 hour pNGAL to be predictive of AKI and hence no further studies of late pNGAL measures are required.

NGAL Assay

Samples were analysed for NGAL protein expression by ELISA in eight of eleven studies[25-27, 29, 31-33, 35], ARCHITECT triage point of care assay in one study[28] and chemiluminescent immunoassay in the remaining two [30, 34]. No strong correlation between assay used and the discriminating ability of NGAL for AKI after liver transplantation was identified in our study.

Sources of Heterogeneity

Bennett *et al.* [17] correlated higher NGAL levels with more severe renal disease whilst Hryniewiecka *et al*. [41] demonstrated NGAL variation with inflammatory parameters, erythrocyte count, haematinics, gender, and age. Age related values may be influenced by comorbidity [42]. Liver transplant patients commonly suffer from significant comorbidity, which could affect the diagnostic value of NGAL.

The majority of studies examined in our review showed good predictive ability for the biomarker across the multiple pathologies leading to the need for liver transplantation. The Model for End-stage Liver Disease score is used to assess severity of chronic liver disease [43]. No correlation was found between MELD score and NGAL levels. None of the examined studies reported the effect of different transplant indications on NGAL levels and future work should address this.

All studies except Lewandowska *et al.* [35] used some exclusion criteria for patients with reduced baseline renal function. This study reported the highest NGAL cut-off value at 1225.3ng/mg uCr, while the rest of the studies reported cut-off values in line with those published in the setting of cardiac surgery and sepsis [44, 45]. Lewandowska *et al.* also reported one of the lower AUROC values of 0.65 for NGAL in the prediction of AKI. Three studies [27] excluded only patients with ESRD and on RRT and achieved good AUROCs of 0.78-0.83. When patients with baseline SCr levels over 1.5 mg/dL were excluded from analysis, pNGAL became a more accurate predictor of post-operative AKI [31].

Plasma NGAL has been shown to be predictive of AKI in patients with CKD undergoing cardiac surgery, though required a higher cut-off value [46]. If NGAL is to enter routine clinical use chosen cut-off values must be validated in populations with baseline renal impairment. Adjustments for baseline values, such as those used by Cheng *et al.* [25] and Khosravi *et al.* [33] could be valuable in off-setting potentially higher baseline values in patients with CKD and co-morbidity.

NGAL rises significantly in sepsis without AKI [47]. Urinary Tract Infection (UTI) also raises urinary NGAL levels. Patients undergoing liver transplantation with established or new infection could therefore confound the use of NGAL in the diagnosis of AKI. Marcelino *et al.*[34] postulated that Familial Amyloid Polyneuropathy patients, which made up a higher proportion in their non-AKI group, could influence rates of UTI, and lower the predictive value of NGAL. However, they did not provide rates of UTI or separate analysis for the NGAL levels in these patients. An assay selective for monomeric urinary NGAL has been reported to be more specific for AKI in UTI and could reduce the confounding effects of sepsis [48].

Wagner *et al.* [27] found LRLT (Living Related Liver Transplant) recipients to have significantly lower uNGAL/uCr rise in post-operative AKI than deceased donor transplants. While they analysed the power of NGAL to differentiate severity of AKI they did not report on AKI severity differences between LRLT and DCD donor groups. Jeong *et al.* [30] examined only LRLT recipients and found NGAL to be predictive of AKI, although with a lower AUROC than Wagner *et al.* [27] (0.69 vs 0.8 respectively). 56% of patients were LRLT recipients in the study by Li *et al.* [29] who reported reasonable predictive value of 0.766. Overall there does not appear to be convincing evidence that NGAL is inferior in predicting AKI in LRLT than DCD recipients however all studies reported on low patient numbers.

Storage of NGAL at -20^{o}C, but not at -80^{o}C, has been reported to produce random decomposition and unpredictable results [49]. Nine of 11 studies presented used -80-^{o}C storage. Marcellino *et al.* [34] used -20^{o}C storage and found poor predictive value for uNGAL in their study . Khosravi *et al.* [33] reported a similarly poor outcome for

pNGAL. This group did not report on the storage temperature in their study, and this could again be a potential confounder. On the other hand Niemann *et al.* [31] also stored samples at -20C reporting an AUROC of 0.79, which was in line with majority of studies. Time in storage before analysis could be a cause of variation, was not reported on by any of the studies and is a factor which requires investigation.

Diagnosing severity of AKI

Wagner *et al.* [27] presented data separately for AKI above RIFLE-R, -I and –F. More severe AKI produced higher values of uNGAL/uCr than less severe AKI. Portal *et al.* [32] also describe a higher pNGAL cut-off value which could differentiate AKIN Stage 1 and below from more severe AKI.

Study Limitations

It was not possible to combine the data in the selected trials due to insufficient data reporting. Future studies investigating NGAL for AKI prediction in OLT must have more complete reporting of data as per the STARD statement [50].

All studies used the Creatinine based definition of AKI, omitting urinary output. This could miss a significant proportion of renal injury and future comparison of NGAL with full AKIN or KDIGO criteria with urinary output measurement should be considered.

All studies reviewed except one [30] used retrospective cut-offs, which could over-estimate the predictive value of NGAL. Prospective cut-off points should be identified and validated before NGAL can enter routine clinical use. Future studies could also consider reporting subgroup analysis by OLT indication, baseline liver function and demographic data, as these will likely impact on baseline NGAL values.

Conclusion

NGAL studies would suggest it to be a valuable early biomarker of AKI after liver transplantation. However, the variability of published studies and the retrospective application of diagnostic cut-off values would suggest that further work is necessary to identify the optimal NGAL source of samples, their storage and assay method, optimal cut off values and source of confounding factors prior to routine clinical use.

References

1. Gainza, F.J., et al., *Evaluation of acute renal failure in the liver transplantation perioperative period: Incidence and impact.* Transplantation Proceedings, 2002. **34**(1): p. 250-251.
2. Barri, Y.M., et al., *Acute Kidney Injury Following Liver Transplantation: Definition and Outcome.* Liver Transplantation, 2009. **15**(5): p. 475-483.
3. Hilmi, I.A., et al., *Acute kidney injury following orthotopic liver transplantation: incidence, risk factors, and effects on patient and graft outcomes.* Br J Anaesth, 2015. **114**(6): p. 919-26.
4. Mehta, R.L., et al., *Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury.* Critical Care, 2007. **11**(2).
5. Bellomo, R., et al., *Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group.* Critical Care, 2004. **8**(4): p. R204.
6. Devarajan, P., *Neutrophil gelatinase-associated lipocalin (NGAL): A new marker of kidney disease.* Scandinavian Journal of Clinical & Laboratory Investigation, 2008. **68**: p. 89-94.
7. Slack, A., A. Yeoman, and J. Wendon, *Renal dysfunction in chronic liver disease.* Critical Care, 2010. **14**(2): p. 214-214.
8. Basile, D.P., M.D. Anderson, and T.A. Sutton, *Pathophysiology of Acute Kidney Injury.* Comprehensive Physiology, 2012. **2**(2): p. 1303-1353.

9. Rabb, H., *Novel urinary markers for early diagnosis of ARF.* American Journal of Kidney Diseases, 2003. **42**(3): p. 599-600.
10. Chakraborty, S., et al., *The Multifaceted Roles of Neutrophil Gelatinase Associated Lipocalin (NGAL) In Inflammation and Cancer.* Biochimica et Biophysica Acta, 2012. **1826**(1): p. 129-169.
11. Supavekin, S., et al., *Differential gene expression following early renal ischemia/reperfusion.* Kidney Int, 2003. **63**(5): p. 1714-24.
12. Kuwabara, T., et al., *Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli, proximal tubules, and distal nephrons.* Kidney International, 2009. **75**(3): p. 285-294.
13. Mori, K., et al., *Endocytic delivery of lipoccalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury.* Journal of Clinical Investigation, 2005. **115**(3): p. 610-621.
14. Mori, K. and K. Nakao, *Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage.* Kidney International, 2007. **71**(10): p. 967-970.
15. Mishra, J., et al., *Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury.* Journal of the American Society of Nephrology, 2003. **14**(10): p. 2534-2543.
16. Mishra, J., et al., *Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery.* Lancet, 2005. **365**(9466): p. 1231-1238.
17. Bennett, M., et al., *Urine NGAL predicts severity of acute kidney injury after cardiac surgery: A prospective study.* Clinical Journal of the American Society of Nephrology, 2008. **3**(3): p. 665-673.
18. Hall, I.E., et al., *IL-18 and Urinary NGAL Predict Dialysis and Graft Recovery after Kidney Transplantation.* Journal of the American Society of Nephrology : JASN, 2010. **21**(1): p. 189-197.
19. Nickolas, T.L., et al., *Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury.* Ann Intern Med, 2008. **148**(11): p. 810-9.
20. Kumpers, P., et al., *Serum neutrophil gelatinase-associated lipocalin at inception of renal replacement therapy predicts survival in critically ill patients with acute kidney injury.* Crit Care, 2010. **14**(1): p. R9.
21. Yang, H.N., et al., *Urine Neutrophil Gelatinase-Associated Lipocalin: An Independent Predictor of Adverse Outcomes in Acute Kidney Injury.* American Journal of Nephrology, 2010. **31**(6): p. 501-509.
22. Ariza, X., et al., *Neutrophil gelatinase-associated lipocalin is a biomarker of acute-on-chronic liver failure and prognosis in cirrhosis.* J Hepatol, 2016. **65**(1): p. 57-65.
23. Firu, S.G., et al., *Neutrophil Gelatinase Associated Lipocalin (NGAL) – a biomarker of renal dysfunction in patients with liver cirrhosis: Do we have enough proof?* Journal of Medicine and Life, 2015. **8**(Spec Issue): p. 15-20.
24. Whiting, P.F., et al., *QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies.* Ann Intern Med, 2011. **155**(8): p. 529-36.
25. Cheng, C.W., et al., *The Ratio of Plasma Neutrophil Gelatinase-Associated Lipocalin Predicts Acute Kidney Injury in Patients Undergoing Liver Transplantation.* Transplantation Proceedings, 2012. **44**(3): p. 776-779.

26. Sirota, J.C., et al., *Urine IL-18, NGAL, IL-8 and serum IL-8 are biomarkers of acute kidney injury following liver transplantation.* Bmc Nephrology, 2013. **14**.
27. Wagener, G., et al., *Urinary neutrophil gelatinase-associated lipocalin as a marker of acute kidney injury after orthotopic liver transplantation.* Nephrology Dialysis Transplantation, 2011. **26**(5): p. 1717-1723.
28. Dedeoglu, B., et al., *Novel biomarkers for the prediction of acute kidney injury in patients undergoing liver transplantation.* Biomarkers in Medicine, 2013. **7**(6): p. 947-957.
29. Li, Y., et al., *Urinary neutrophil gelatinase-associated lipocalin and L-type fatty acid binding protein as diagnostic markers of early acute kidney injury after liver transplantation.* Biomarkers, 2012. **17**(4): p. 336-342.
30. Jeong, T.-D., et al., *Neutrophil gelatinase-associated lipocalin as an early biomarker of acute kidney injury in liver transplantation.* Clinical Transplantation, 2012. **26**(5): p. 775-781.
31. Niemann, C.U., et al., *Acute Kidney Injury During Liver Transplantation as Determined by Neutrophil Gelatinase-Associated Lipocalin.* Liver Transplantation, 2009. **15**(12): p. 1852-1860.
32. Portal, A.J., et al., *Neutrophil Gelatinase-Associated Lipocalin Predicts Acute Kidney Injury in Patients Undergoing Liver Transplantation.* Liver Transplantation, 2010. **16**(11): p. 1257-1266.
33. Khosravi, M.B., S. Milani, and F. Kakaei, *Serum Neutrophil Gelatinase-Associated Lipocalin versus Serum Creatinine for the Prediction of Acute Kidney Injury after Liver Transplantation.* International journal of organ transplantation medicine, 2013. **4**(3): p. 102-9.
34. Marcelino, P., et al., *Is Urinary gamma-Glutamyl Transpeptidase Superior to Urinary Neutrophil Gelatinase-Associated Lipocalin for Early Prediction of Acute Kidney Injury After Liver Transplantation?* Transplantation Proceedings, 2014. **46**(6): p. 1812-1818.
35. Lewandowska, L., et al., *Netrin-1 and Semaphorin 3A Predict the Development of Acute Kidney Injury in Liver Transplant Patients.* Plos One, 2014. **9**(10).
36. Zarbock, A., et al., *Effect of Early vs Delayed Initiation of Renal Replacement Therapy on Mortality in Critically Ill Patients With Acute Kidney Injury: The ELAIN Randomized Clinical Trial.* JAMA, 2016. **315**(20): p. 2190-9.
37. Au, V., et al., *Urinary neutrophil gelatinase-associated lipocalin (NGAL) distinguishes sustained from transient acute kidney injury after general surgery.* KI Rep, 2016. **1**(1): p. 3-9.
38. Haase, M., et al., *The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies.* J Am Coll Cardiol, 2011. **57**(17): p. 1752-61.
39. Helmersson-Karlqvist, J., J. Ärnlöv, and A. Larsson, *Day-to-day variation of urinary NGAL and rational for creatinine correction.* Clinical Biochemistry, 2013. **46**(1–2): p. 70-72.
40. Umbro, I., et al., *Acute kidney injury and post-reperfusion syndrome in liver transplantation.* World Journal of Gastroenterology, 2016. **22**(42): p. 9314-9323.

41. Hryniewiecka, E., et al., *Is Neutrophil Gelatinase-Associated Lipocalin an Optimal Marker of Renal Function and Injury in Liver Transplant Recipients?* Transplantation Proceedings, 2014. **46**(8): p. 2782-2785.
42. Haase, M., et al., *Accuracy of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Diagnosis and Prognosis in Acute Kidney Injury: A Systematic Review and Meta-analysis.* American Journal of Kidney Diseases, 2009. **54**(6): p. 1012-1024.
43. Wiesner, R., et al., *Model for end-stage liver disease (MELD) and allocation of donor livers.* Gastroenterology, 2003. **124**(1): p. 91-6.
44. Zhang, A., et al., *Diagnosis and prognosis of neutrophil gelatinase-associated lipocalin for acute kidney injury with sepsis: a systematic review and meta-analysis.* Crit Care, 2016. **20**: p. 41.
45. Zhou, F., et al., *Diagnostic value of neutrophil gelatinase-associated lipocalin for early diagnosis of cardiac surgery-associated acute kidney injury: a meta-analysis.* Eur J Cardiothorac Surg, 2016. **49**(3): p. 746-55.
46. Doi, K., et al., *Plasma neutrophil gelatinase-associated lipocalin in acute kidney injury superimposed on chronic kidney disease after cardiac surgery: a multicenter prospective study.* Critical Care, 2013. **17**(6): p. R270.
47. Otto, G.P., et al., *Impact of sepsis-associated cytokine storm on plasma NGAL during acute kidney injury in a model of polymicrobial sepsis.* Crit Care, 2013. **17**(2): p. 419.
48. Cai, L., et al., *The Origin of Multiple Molecular Forms in Urine of HNL/NGAL.* Clinical Journal of the American Society of Nephrology : CJASN, 2010. **5**(12): p. 2229-2235.
49. Haase-Fielitz, A., M. Haase, and R. Bellomo, *Instability of urinary NGAL during long-term storage.* Am J Kidney Dis, 2009. **53**(3): p. 564-5; author reply 566.
50. Bossuyt, P.M., et al., *STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies.* BMJ : British Medical Journal, 2015. **351**.

Figure 1. Flowchart showing study search and selection process

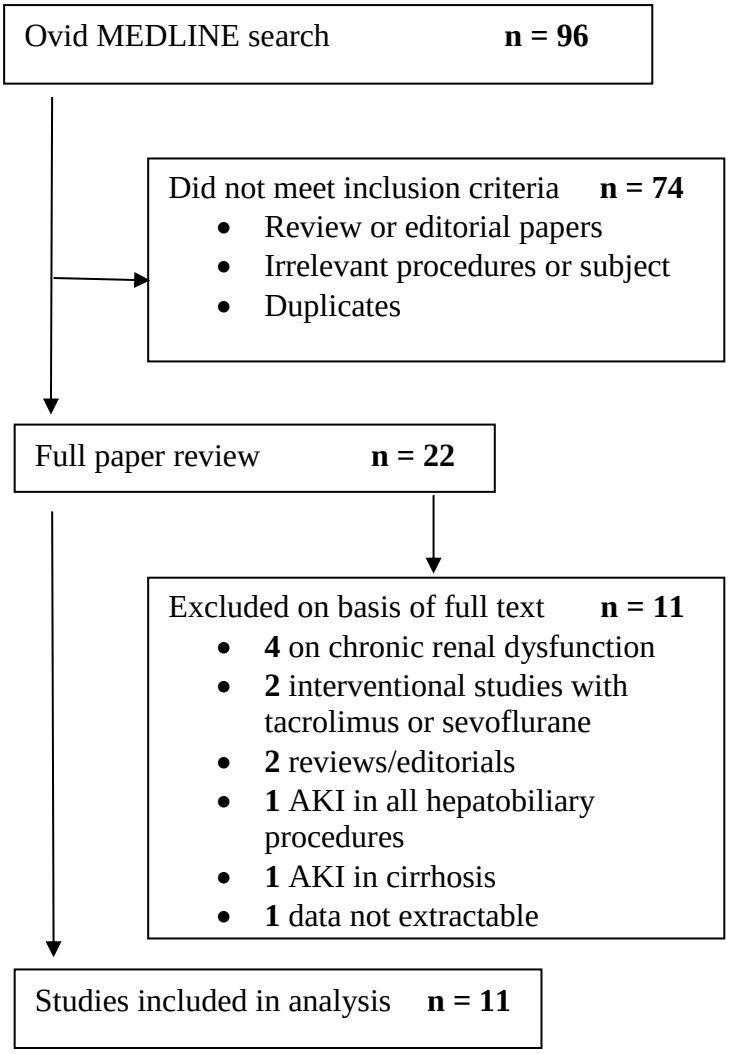

Table 1. Study Demographics

Reference	Country	Design	Centres	Patients	Male %	Av. Age	% LRD	MELD	AKI Definition	% AKI
Niemann 2009	USA	Prospective	2	44	66.7	54	N/A	21(+/-10)	RIFLE Cr	53.3
Portal 2010	UK	Prospective	1	80	58.9	50	N/A	16(12-28)	AKIN Cr	37.5
Wagner 2011	USA	Prospective	1	92	65.2	54.3	19.6	21(+/-7.4)	RIFLE Cr	40.2
Cheng 2012	Taiwan	Prospective	1	26	76.9	56.5	N/A	16.7(+/-8.9)	AKIN Cr	50
Jeong 2012	Korea	Prospective	1	19	78.9	52	100	18.1(+/-8.9)	RIFLE Cr	57.9
Li 2012	China	Prospective	1	25	88.0	47	56	8.2(3.9-13.3)	AKIN Cr	44.0
Dedeoglu 2013	Netherlands	Prospective	1	26	65.4	54	N/A	NR	AKIN Cr	34.6
Khosravi 2013	Iran	Prospective	1	90	66.7	40.2	N/A	22(+/-6.2)	Serum Cr >0.3mg/dL	34.4
Sirota 2013	USA	Prospective	2	40	67.5	56.2	N/A	18.2(NR)	RIFLE Cr "R" > 24 hours	17.5
Lewandowska 2014	Poland	Prospective	1	63	68.3	46	N/A	NR	RIFLE Cr	55.6
Marcelino 2014	Portugal	Prospective	1	61	45.9	49	N/A	13.3(NR)	AKIN Cr	31.1

Av. Age - average age, MELD - Model for End-Stage Liver Disease score, %LRD - percentage living related donors, AKI - Acute Kidney Injury, RIFLE Cr - RIFLE Classification creatinine criteria "Risk" and over, AKIN Cr - Acute Kidney Injury Network Classification creatinine criteria Stage 1 and over, Serum Cr >0.3mg/dL - Creatinine change over 0.3mg/dL, RIFLE "R" > 24 hours - Rifle "R" and over for over 24 hours, N/A - Not Applicable, NR - Not Reported

Table 2. Quality assessment of included studies uisng QUADAS-2

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Timing	Patient Selection	Index Test	Reference Standard
Niemann *et al* 2009	☺	☹*c,d*	☺	☹*g*	☺	☺	☺
Portal *et al.* 2010	?*a*	☹*c*	☺	?*h*	☺	☺	☺
Wagner *et al.* 2011	☺	☹*c*	☺	☺	☺	☺	☺
Cheng et al. 2012	?*a*	☹*c*	☺	? *i*	☺	☺	☺
Jeong *et al.* 2012	?*a*	☺	☺	☺	?*j*	☺	☺
Li *et al.* 2012	☺	☹*c*	☺	☺	☺	☺	☺
Dedeoglu *et al.* 2013	☺	☹*c*	☺	☺	☺	☺	☺
Khosravi *et al.* 2013	☹*b*	☹*c,e*	?*f*	☺	☺	☺	☺
Sirota *et al.* 2013	☺	☹*c*	☺	☺	☺	☺	☺
Lewandowska *et al.* 2014	☺	☹*c*	☺	☺	☺	☺	☺
Marcelino *et al.* 2014	☺	☹*c,d*	☺	☺	☺	☺	☺

QUADAS-2 - Quality Assessment of Diagnostic Accuracy Studies 2, ☺ low risk, ☹ *h* igh risk, *?* unclear risk; *a* - unknown if a consecutive or random patient sample enrolled; *b* - broad exclusion criteria; *c* - defined threshold retrospectively; *d* - sample storage at -20; *e* - unknown storage temperature; *f* - used 0.3mg/dL creatinine change only as reference standard; *g* - fourteen patients with Cr>1.5mg/dL excluded after recruitment; *h* - urine samples from only 46 of 80 patients obtained; *i* - did not analyse 4 diceased patients, unclear time of death; * - living related donor transplant recipients only;

Table 3 Results for Studies on Plasma NGAL

Reference	Patients	AKI Definition	% AKI	Assay	Cutoff (mg/dL)	Storage	Baseline	Best Time (hr)	Sensitivity	Specificity	AUROC	p value
Plasma NGAL												
Niemann 2009	45	RIFLE Cr > R	53.3	ELISA	139	-	Reperfusion	2	0.67	0.8	0.79	0.0004
Portal 2010	80	AKIN > Stage 1	37.5	ELISA	212	-	ITU Admission	12	0.67	0.84	0.79	0.29
Dedeoglu 2013	26	AKIN > Stage 1	34.6	Triage POC	243	-	ITU Admission	4 8	NR NR	NR NR	0.84 0.86	0.00 0.86
Khosravi 2013	90	Serum Cr >	34.4	ELISA	0.57	NR	Reperfusion	2	NR	NR	0.54	NR
Jeong	19	RIFLE Cr > R	57.9	NGAL Test	50% baseline	-	Reperfusion	2	NR	NR	0.68	0.18
Plasma NGAL divided by baseline NGAL												
Cheng 2012	22	AKIN > Stage 1	59.1	ELISA	NR	-	Reperfusion	1	NR	NR	0.78	0.02
Plasma NGAL minus baseline												
Khosravi 2013	90	Serum Cr >	34.4	ELISA	NR	NR	Reperfusion	2	0.59	0.71	0.64	NR

Table 3. Details of reviewed studies with reported diagnostic accuracy sensitivity and specificity Abbreviations: NR (Not Reported); AKIN (Acute Kidney Injury Network Classification); RIFLE (Risk Injury Failure Loss End-Stage Classification) Cr (Creatinine); ELISA (Enzyme Linked Immunosorbent Assay); Triage POC (Alere Triage Point-of-Care Assay); NGAL Test (BioPorto Particle Enhanced Turbidimetric Assay); ARCHITECT (Abbott Chemiluminescent Microparticle

Table 4. Results for studies on Urinary NGAL

Reference	Patients	AKI Definition	% AKI	Assay	Cutoff (ng/mL)	Storage	Baseline	Best Time (hr)	Sensitivity	Specificity	AUROC	p value
Urinary NGAL												
Portal 2010	46	AKIN Cr > Stage 1	37.5	ELISA	150	-80	ITU Admission	12	NR	NR	0.76	0.01
Dedeoglu 2013	26	AKIN Cr > Stage 1	34.6	Triage POC	103.5	-80	ITU Admission	0	NR	NR	0.79	0.023
					90			4	NR	NR	0.8	0.012
					94.5			8	NR	NR	0.76	0.041
Sirota 2013	40	RIFLE Cr "R" > 24 hrs	17.5	ELISA	NR	-80	End of Surgery	24	NR	NR	0.83	NR
Marcelino 2014	61	AKIN Cr > Stage 1	31.1	ARCHITECT	44.6	-20	Reperfusion	12	35.7	84.2	0.5	NR

Table 4. Details of reviewed studies with reported diagnostic accuracy sensitivity and specificity. NR (Not Reported); AKIN (Acute Kidney Injury Network Classification); RIFLE (Risk Injury Failure Loss End-Stage Classification) Cr (Creatinine); ELISA (Enzyme Linked Immunosorbent Assay); Triage POC (Alere Triage Point-of-Care Assay); NGAL Test (BioPorto Particle Enhanced Turbidimetric Assay); ARCHITECT (Abbott Chemiluminescent Microparticle Immunoassay)

Table 5. Results for studies on Urinary NGAL corrected for Urinary Creatinine

Reference	Patients	AKI Definition	% AKI	Assay	Cutoff (ng/mguCr)	Storage	Baseline	Best Time (hr)	Sensitivity	Specificity	AUROC	p value
Urinary NGAL corrected for urinary Creatinine												
Lewandowska 2014	63	RIFLE Cr > R	55.6	ELISA	1225.3	-80	End of Surgery	2	0.51	0.89	0.65	0.0306
Li 2012	25	AKIN Cr > Stage 1	44	ELISA	43.02	-80	Reperfusion	2	0.727	0.786	0.766	0.025
					26.97			4	0.636	0.857	0.773	0.021
					17.19			6	0.727	0.714	0.773	0.021
Wagner 2011	92	RIFLE Cr > R	40.2	ELISA	74	-80	Reperfusion	3	0.84	0.68	0.8	<0.0001
								18	0.636	0.551	0.72	<0.005
Jeong 2012	19	RIFLE Cr > R	57.99	ARCHITECT	50% baseline	-70	Reperfusion	2	NR	NR	0.69	0.16
Marcelino 2014	61	AKIN Cr > Stage 1	31.1	ARCHITECT	44.6	-20	Reperfusion	12	NR	NR	0.407	NR

Table 5. Details of reviewed studies with reported diagnostic accuracy sensitivity and specificity and forest plot of Area Under Receive-Operator Characteristic with 95% confidence intervals. Abbreviations: NR (Not Reported); AKIN (Acute Kidney Injury Network Classification); RIFLE (Risk Injury Failure Loss End-Stage Classification) Cr (Creatinine); ELISA (Enzyme Linked Immunosorbent Assay); Triage POC (Alere Triage Point-of-Care Assay); NGAL Test (BioPorto Particle Enhanced Turbidimetric Assay); ARCHITECT (Abbott Chemiluminescent Microparticle Immunoassay)