

RESEARCH ARTICLE

# Comparison of macrophage migration inhibitory factor and neutrophil gelatinase-associated lipocalin-2 to predict acute kidney injury after liver transplantation: An observational pilot study

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## Abstract

### Introduction

Several biomarkers have been suggested as early predictors of acute kidney injury (AKI) after orthotopic liver transplantation (OLT). Neutrophil gelatinase-associated lipocalin-2 (NGAL) appears to be a promising predictor of AKI after OLT, but the clinical benefit remains to be proven. Recently, systemic macrophage migration inhibitory factor (MIF) has been proposed as early indicator for requirement of renal replacement therapy after OLT. The aim of this prospective, observational pilot study was to compare the predictive values of serum and urinary MIF for severe AKI after OLT to those of serum and urinary NGAL.

### Methods

Concentrations of MIF and NGAL were measured in serum and urine samples collected from patients undergoing OLT. Acute kidney injury was classified according to the KDIGO criteria, with stages 2 and 3 summarized as severe AKI. Areas under the receiver operating curves (AUC) were calculated to assess predictive values of MIF and NGAL for the development of severe AKI.

### Results

Forty-five patients (mean age 55±8 years) were included. Nineteen patients (38%) developed severe AKI within 48 hours after reperfusion. At the end of OLT, serum MIF was predictive of severe AKI (AUC 0.73; 95% confidence intervals, CI 0.55–0.90; P = 0.03), whereas urinary MIF, serum NGAL, and urinary NGAL were not. On the first postoperative day, serum MIF (AUC 0.78; CI 0.62–0.93; P = 0.006), urinary MIF (AUC 0.71; CI 0.53–0.88;

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$P = 0.03$ ), and urinary NGAL (AUC 0.79; CI 0.64–0.93;  $P = 0.02$ ) were predictive for severe AKI, while serum NGAL was not.

## Conclusion

In the setting of OLT, MIF and NGAL had similar predictive values for the development of severe AKI.

## Introduction

Acute kidney injury (AKI) is a major complication after orthotopic liver transplantation (OLT) associated with increased morbidity and mortality, reduced graft survival, and prolonged hospital length-of-stay [1–3]. Up to 50% of patients undergoing OLT develop AKI [1,4–7]. Approximately 30% of patients with AKI require renal replacement therapy (RRT) following OLT [3,7,8]. Current management of AKI includes optimization of the fluid status and dose-adjustment of nephrotoxic drugs. Recent studies suggest that early institution of RRT may improve survival in patients with severe AKI [9,10], emphasizing the need for early recognition of postoperative AKI.

Acute kidney injury is diagnosed by evaluating changes in serum creatinine (sCr) concentrations and urine output [11]. Serum creatinine is a poor marker of AKI in patients with end-stage liver disease, as sCr concentrations may be influenced by decreased creatinine biosynthesis, reduced muscle mass, and increased serum bilirubin concentrations [12]. Additionally, sCr concentrations increase when renal injury is already present, limiting their use for early detection of AKI [13]. Systemic and urinary neutrophil gelatinase-associated lipocalin-2 (NGAL) have been suggested as promising biomarkers for early prediction of AKI in patients undergoing OLT [4,14–16]. However, as the clinical value of NGAL remains to be proven, new molecules are being investigated for their potential to predict AKI after OLT.

Recently, the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) has been associated with AKI in humans. Plasma MIF concentrations were independently associated with the severity of AKI in critically ill patients [17]. Furthermore, we have reported that systemic MIF has a good prognostic value to identify patients requiring postoperative RRT after OLT [7]. In addition, urinary MIF has been proposed as a predictor of AKI, particularly in patients with inflammatory nephritis [18,19] and kidney transplant rejection [20]. However, whether systemic or urinary MIF can predict the development of AKI after OLT with comparable power as systemic and urinary NGAL remains to be elucidated. In this current study we compared the predictive values of serum and urinary MIF for the development of severe AKI after OLT to those of serum and urinary NGAL.

## Patients and methods

### Study subjects

This single-center, prospective, observational pilot study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki and the Declaration of Istanbul. Institutional ethics committee approval for the study was obtained (Ethikkommission der Medizinischen Universität Wien, reference number 1271/2014), and the study was registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT02695979). None of the transplant donors were from a vulnerable population. All patients provided written informed consent before inclusion.

Consecutive patients with end-stage liver disease scheduled for OLT at the General Hospital of Vienna between August 2014 and August 2015 were enrolled in the study. Preoperative kidney function was assessed by evaluating sCr concentrations measured at hospital admission immediately before OLT. Estimated glomerular filtration rate (eGFR) was calculated according to the 'Modification of Diet in Renal Disease Study' Equation [21]. Patients with severe preoperative kidney dysfunction ( $eGFR < 30 \text{ mL/min/1.73 m}^2$ ) were not enrolled in the study. Other exclusion criteria were combined liver-lung or liver-kidney transplantation, high urgency transplantation, and the requirement of veno-venous bypass during OLT.

## Anesthesia, surgery, and immunosuppression

Orthotopic liver transplantations were performed under general anesthesia using the local standard technique with cross-clamping of the caval vein. After transplantation, all patients were admitted to the intensive care unit (ICU). Immunosuppression with intravenous application of 40 mg dexamethasone was initiated before graft reperfusion. The second dose of intravenous dexamethasone (32 mg) was administered 24 hours after reperfusion, and was further reduced daily by 8 mg until reaching the maintenance dose of 8 mg. Additionally, postoperative immunosuppression was induced with anti-thymocyte globulin (2.5 kg per kg body weight) within two hours after arrival at the ICU and maintained with low-dose tacrolimus starting on the fourth postoperative day (trough level 6–8 ng/ml).

## Data and sample collection

Epidemiological patient data were collected prior to surgery. The model for end-stage liver disease (MELD) score was calculated preoperatively to assess the severity of liver disease. Perioperative data including laboratory values, duration of surgery, cold ischemia time, caval clamping time, blood loss, transfusion of packed red blood cells, platelets and fresh frozen plasma, administration of coagulation factors, fluid balance, and urine output were recorded for one week following OLT. Data were extracted from the patient data management system (ICCA, Phillips Healthcare, Hamburg, GER). Serum and urine samples were collected at the following time points: at baseline (BL) during stable hemodynamic conditions after induction of anesthesia, prior to surgical skin incision; at the end of surgery (day 0); at 24 hours after reperfusion on the first postoperative day (day 1); and at 48 hours after reperfusion on the second postoperative day (day 2). Serum samples were sent to the central laboratory for analysis of blood chemistry, including sCr. For measurement of MIF and NGAL concentrations, serum and urine samples were centrifuged for 30 minutes at 1000 g and supernatants were stored at  $-80^\circ\text{C}$  until analysis.

## Diagnosis and classification of AKI

Acute kidney injury was diagnosed and classified according to the KDIGO criteria (stage 0, 1, 2 and 3 AKI) [11]. Serum creatinine concentrations obtained from samples collected 48 hours after reperfusion (day 2) were compared to pre-operative sCr concentrations to diagnose AKI and classify the AKI stage. Patients met the criteria for stage 0 AKI (i.e. no AKI) if differences between pre-operative sCr concentrations and sCr concentrations on day 2 were below 0.3 mg/dl. Criteria for stage 1 AKI were met when sCr concentrations on day 2 were at least 0.3 mg/dl greater than pre-operative sCr concentrations. A 2-fold to 3-fold increase of sCr concentrations on day 2 from pre-operative sCr concentrations characterized stage 2 AKI. Criteria for stage 3 AKI were met when the sCr concentrations on day 2 were at least 3-fold compared to pre-operative sCr concentrations, or when sCr was above 4 mg/dl. Individuals receiving RRT within 48 hours after reperfusion were considered to have met the criteria for stage 3

AKI. After AKI classification, patients were separated into two groups: Patients developing no AKI or stage 1 AKI at day 2 after OLT were summarized as the no/mild AKI group, whereas patients meeting the criteria for stage 2 or 3 AKI at day 2 after OLT were summarized as the severe AKI group.

## MIF and NGAL concentrations and prediction of AKI

Enzyme-linked immunosorbent assays were used according to the manufacturer's protocol to determine concentrations of MIF (human MIF Quantikine kit; R&D Systems, Minneapolis, MN) and NGAL (human Lipocalin-2 ELISA; RayBiotech, Norcross, GA) in serum and urine samples collected at baseline, day 0, day 1 and at day 2.

In order to assess whether MIF and NGAL could predict AKI prior to conventional diagnosis of AKI using the KDIGO criteria, the predictive performance of MIF and NGAL was analyzed separately for day 0 and day 1. The discriminatory power to predict severe AKI was analyzed for serum MIF, serum NGAL, urinary MIF, and urinary NGAL, and compared between patients in the no/mild AKI group and those in the severe AKI group. In addition, the predictive power of MIF and NGAL for the development of any AKI was assessed by comparing values between patients not developing AKI and those developing any stage of AKI. Furthermore, we assessed the performance of MIF and NGAL to diagnose the development of AKI on day 2, which was the time point used to diagnose AKI by the KDIGO criteria.

## Statistical analysis

Statistical analyses were performed using Prism 6.0 (GraphPad Software, La Jolla, CA). Results are depicted as median with interquartile ranges (IQR 25%-75%), while continuous variables are expressed as mean  $\pm$  standard deviation. Two-way ANOVA with Bonferroni correction was used to compare differences among groups at various time points. Differences within groups at various time points were analyzed with an unpaired t-test and corrected for multiple comparisons with the Holm-Sidak method. In order to determine the true positive rate in function of the false positive rate at different cut-off points, a receiver operating characteristics (ROC) curve analysis was performed for MIF and NGAL in serum and in urine as predictors of severe AKI. An area under the curve (AUC) of 0.90–1.0 indicated excellent, 0.80–0.89 good, 0.70–0.79 fair, 0.60–0.69 poor, and 0.50–0.59 no useful predictive value for the development of AKI [22]. For all statistical analyses, an adjusted P-value  $<0.05$  was considered significant. Adjusted P values are reported when data have been corrected for multiple comparisons.

Sample size was calculated based on data from our previous study [7]. Calculation for an area under ROC curve of 0.75 with type I error of 0.05 and type II error of 0.2 (power 80%) estimated a sample size of 45 patients.

## Results

### Patient characteristics and incidence of AKI after OLT

Forty-five patients were enrolled in the study. Demographic data of the study population and the etiology of liver disease are listed in Table 1. On day 2 after OLT, 15 patients (33%) met the criteria for stage 1 AKI, 9 patients (20%) were diagnosed stage 2 AKI, and 10 patients (22%) met the criteria for stage 3 AKI. There were no differences in preoperative sCr concentrations, glomerular filtration rate, body-mass index, MELD score, or intraoperative transfusion requirements among the no/mild AKI group and the severe AKI group (Table 2). There was no difference in sCr concentrations between patients without severe AKI and those with severe AKI on day 0. As sCr was used to diagnose AKI, sCr concentrations were greater in patients

**Table 1. Demographic data of the study population.**

Sex	
Male, n (%)	36 (80)
Female, n (%)	9 (20)
Ethnicity	European
Etiology of end-stage liver disease	
Alcohol-induced, n (%)	22 (49)
Viral, n (%)	9 (20)
Hepatocellular carcinoma, n (%)	4 (9)
Autoimmune, n (%)	4 (9)
Primary biliary cirrhosis, n (%)	2 (4)
Other, n (%)	4 (9)

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developing severe AKI than in patients without AKI on day 1 and day 2 after OLT (“S1 Fig”). Furthermore, cold ischemia time and caval clamping time did not differ among groups. No major complications such as massive postoperative hemorrhage (requirement of >4 units of packed red blood cells over 48 hours), sepsis, hepatic artery occlusion, and caval vein thrombosis were observed in any patient during the ICU stay.

### Serum concentrations of MIF and NGAL in patients with severe AKI

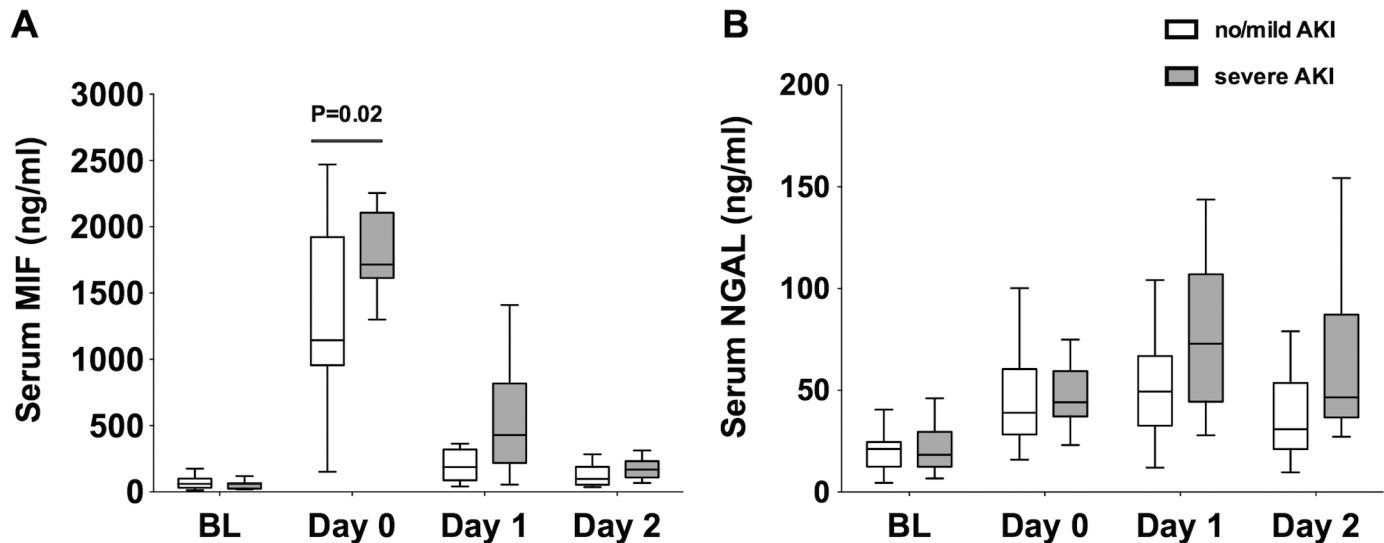
Baseline serum MIF concentrations did not differ among the no/mild AKI group (60 ng/ml, IQR 31–100) and the severe AKI group (60 ng/ml, IQR 27–66,  $P = 0.90$ ; “Fig 1A”). At the end of surgery (day 0), serum MIF concentrations increased in both groups ( $P < 0.001$ ), but were lower in the no/mild AKI group (1143 ng/ml, IQR 955–1922) than in the severe AKI group (1715 ng/ml, IQR 1613–2106,  $P = 0.02$ ). At day 1 after OLT, serum MIF concentrations returned to baseline values in the no/mild AKI group (187 ng/ml, IQR 87–320,  $P = 0.50$  vs. baseline), but remained elevated in the severe AKI group (429 ng/ml, IQR 218–818,  $P = 0.007$  vs. baseline). At day 2 after OLT, serum MIF concentrations did not differ from baseline values in the no/mild AKI group (98 ng/ml, IQR 54–189,  $P = 0.76$  vs. baseline) or in the severe AKI group (169 ng/ml, IQR 109–232,  $P = 0.28$  vs. baseline).

**Table 2. Perioperative characteristics of patients undergoing OLT.**

	All patients	No/mild AKI	Severe AKI	P value
Patients (n)	45	26	19	
Age (years)	55±9	54±8	56±10	0.37
MELD	17±6	16±4	18±7	0.23
eGFR (mL/min/1.73 m <sup>2</sup> )	96±37	85±32	111±39	0.42
Preoperative sCr (mg/dl)	0.9±0.3	1.0±0.3	0.8±0.3	0.41
Body-mass index	26±5	27±4	25±5	0.45
Cold ischemia time (min)	345±148	354±186	332±60	0.68
Caval clamping time (min)	88±22	88±26	88±16	0.99
PRBC units transfused, n	3±3	3±3	4±4	0.14
FFP units transfused, n	6±6	6±6	7±7	0.46
Thrombocyte units transfused, n	1±1	1±1	1±1	0.75

Data are depicted as mean ± standard deviation. P values indicate differences among the no/mild AKI and the severe AKI group. Abbreviations: AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; FFP, fresh frozen plasma; MELD, model for end stage liver disease; PRBC, packed red blood cell; sCr, serum creatinine.

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**Fig 1. Serum concentrations of MIF and NGAL.** Concentration of (A) serum MIF and (B) serum NGAL at 4 different time points: baseline (BL; under anesthesia before skin incision), day 0 (at the end of surgery), day 1 (24 hours after graft reperfusion on day 1 after OLT), and on day 2 (48 hours after reperfusion on day 2 after OLT). White bars indicate values of patients with no AKI or stage 1 AKI, gray bars represent values of patients who developed stage 2 or 3 AKI after undergoing OLT. P values indicate significant differences between groups.

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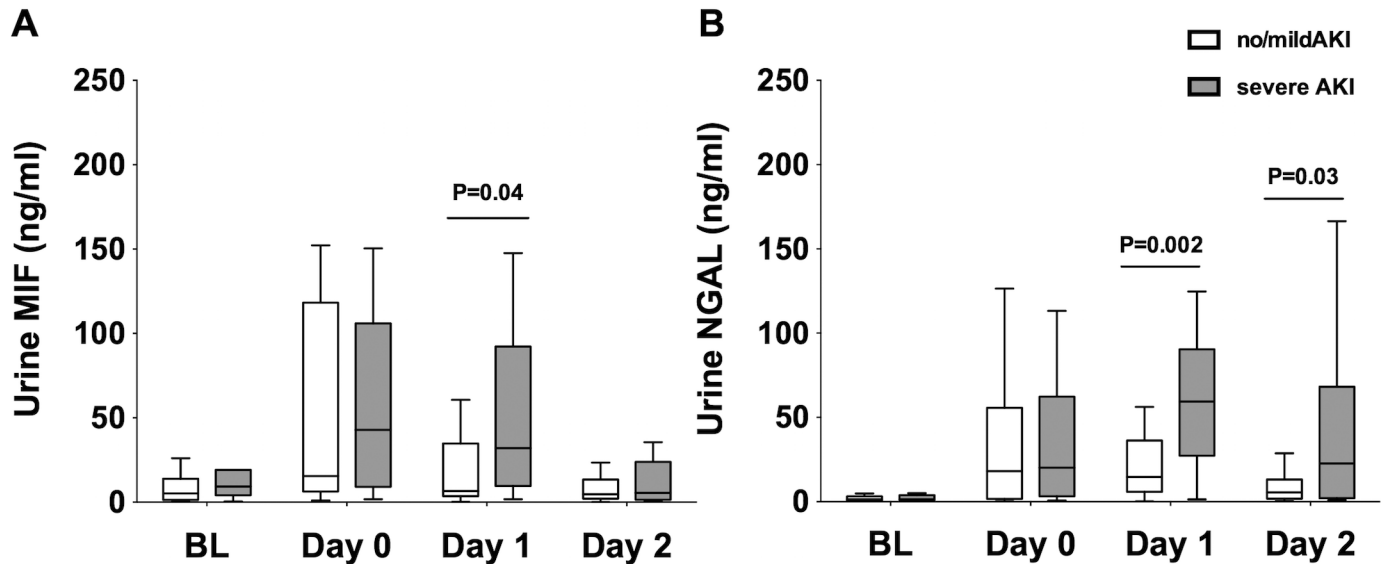
Serum NGAL concentrations at baseline did not differ among the no/mild AKI group (21 ng/ml, IQR 13–25) and the severe AKI group (18 ng/ml, IQR 12–30,  $P > 0.99$ ; “Fig 1B”). At the end of OLT (day 0), serum NGAL concentrations did not differ from baseline in the no/mild AKI group (39 ng/ml, IQR 28–60,  $P = 0.08$ ) or in the severe AKI group (44 ng/ml, IQR 37–59,  $P = 0.10$ ), and did not differ among groups ( $P > 0.99$ ). At day 1 after OLT, serum NGAL concentrations increased to 49 ng/ml, IQR 33–67 in the no/mild AKI group ( $P = 0.005$ ) and to 73 ng/ml, IQR 44–107 in the severe AKI group ( $P < 0.001$ ), but did not differ among groups ( $P = 0.73$ ). At day 2 after OLT, serum NGAL concentrations returned to baseline values in the no/mild AKI group (31 ng/ml, IQR 21–54,  $P = 0.44$ ), but remained elevated in the severe AKI group (47 ng/ml, IQR 37–87  $P = 0.004$  vs. baseline).

### Urinary concentrations of MIF and NGAL in patients with severe AKI

Urine MIF concentrations at baseline did not differ among patients with no/mild AKI (5 ng/ml, IQR 1.4–14) and those with severe AKI (9 ng/ml, IQR 4–19,  $P = 0.87$ ; “Fig 2A”). At the end of OLT (day 0), urine MIF concentrations increased to 15 ng/ml, IQR [6–118] in the no/mild AKI group ( $P = 0.003$ ) and to 43 ng/ml, IQR 9–106 in the severe AKI group ( $P = 0.02$ ), but did not differ among groups ( $P = 0.95$ ). At day 1, urine MIF concentrations were lower in the no/mild AKI group (6.5 ng/ml, IQR 4–35) than in the severe AKI group (32 ng/ml, IQR 9.5–92,  $P = 0.04$ ). At day 2 after OLT, urine MIF concentrations returned to baseline values in both groups.

Urinary NGAL concentrations at baseline did not differ among patients with no/mild AKI (1.7 ng/ml, IQR 0.7–3) and those with severe AKI (1.3 ng/ml, IQR 0.8–2.8,  $P = 0.99$ ; “Fig 2B”). At the end of OLT (day 0), urinary NGAL concentrations increased to 18 ng/ml, IQR 1.6–56 in the no/mild AKI group ( $P < 0.001$ ) and to 20 ng/ml, IQR 3.2–62 in the severe AKI group ( $P = 0.009$ ). Urinary NGAL concentrations at the end of OLT did not differ among groups ( $P = 0.98$ ). At day 1, urine NGAL concentrations remained elevated in patients with no/mild AKI (15 ng/ml, IQR 6–36,  $P = 0.002$ ) and in patients with severe AKI (59 ng/ml, IQR 27–90,





**Fig 2. Urine concentrations of MIF and NGAL.** Concentrations of (A) urinary MIF and (B) urinary NGAL at 4 different time points: baseline (BL; under anesthesia before skin incision), day 0 (at the end of surgery), day 1 (24 hours after graft reperfusion on day 1 after OLT), and on day 2 (48 hours after reperfusion day 2 after OLT). White bars indicate values of patients with no AKI or stage 1 AKI, gray bars represent values of patients who developed stage 2 or 3 AKI after undergoing OLT. P values indicate significant differences between groups.

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$P < 0.001$ ). Urine NGAL concentrations at day 1 were lower in patients with no/mild AKI than in patients with severe AKI ( $P = 0.002$ ). At day 2 after OLT, urine NGAL concentrations returned to baseline values in the no/mild AKI group (5 ng/ml, IQR 1.8–13,  $P = 0.06$  vs. baseline), but remained elevated in the severe AKI group (23 ng/ml, IQR 2–68,  $P = 0.01$  vs. baseline).

### Performance of MIF and NGAL for predicting the development of AKI after OLT

In order to evaluate the predictive value of serum MIF, serum NGAL, urinary MIF, and urinary NGAL for the development of severe AKI after OLT, ROC curve analyses were performed. At the end of OLT, serum MIF was a fair predictor for severe AKI after OLT, whereas serum NGAL, urinary MIF, and urinary NGAL were not predictive for severe AKI (Table 3 and “Fig 3”). On day 1, serum MIF, urinary MIF, and urinary NGAL had fair predictive performance, whereas serum NGAL was a poor predictor for severe AKI. On day 2, serum MIF and serum NGAL had a fair value to diagnose severe AKI, whereas urinary NGAL had poor diagnostic value. Urinary MIF had no discriminatory power for detection of severe AKI at day 2.

Finally, we evaluated the predictive value of serum MIF, serum NGAL, urinary MIF, and urinary NGAL for the development of any stage of AKI after OLT. Serum MIF and serum NGAL did not predict the development of AKI at the end of OLT, but predicted AKI with good accuracy at day 1. Furthermore, serum MIF and serum NGAL had good value to diagnose AKI any stage at day 2 after OLT (Table 4). Urinary MIF and urinary NGAL did not adequately predict nor diagnose the development of AKI after OLT at any of the time points assessed.

### Discussion

In the current study, we serially measured serum and urine concentrations of MIF and NGAL in patients undergoing OLT, and compared the ability of these parameters to predict the

**Table 3. Area under the ROC curve for development of severe AKI.**

	Serum MIF	Serum NGAL	Urine MIF	Urine NGAL
Day 0				
AUC	0.73	0.59	0.58	0.50
95% CI	0.55–0.90	0.41–0.77	0.40–0.75	0.33–0.68
P Value	0.03	0.34	0.40	0.98
Day 1				
AUC	0.78	0.68	0.71	0.79
95% CI	0.62–0.93	0.50–0.85	0.53–0.88	0.64–0.93
P Value	0.006	0.06	0.028	0.002
Day 2				
AUC	0.71	0.75	0.58	0.65
95% CI	0.54–0.89	0.60–0.90	0.37–0.78	0.47–0.84
P Value	0.03	0.009	0.43	0.08

Abbreviations: AKI, acute kidney injury; AUC, area under the curve; CI, confidence intervals; MIF, macrophage migration inhibitory factor; NGAL, neutrophil gelatinase-associated lipocalin-2.

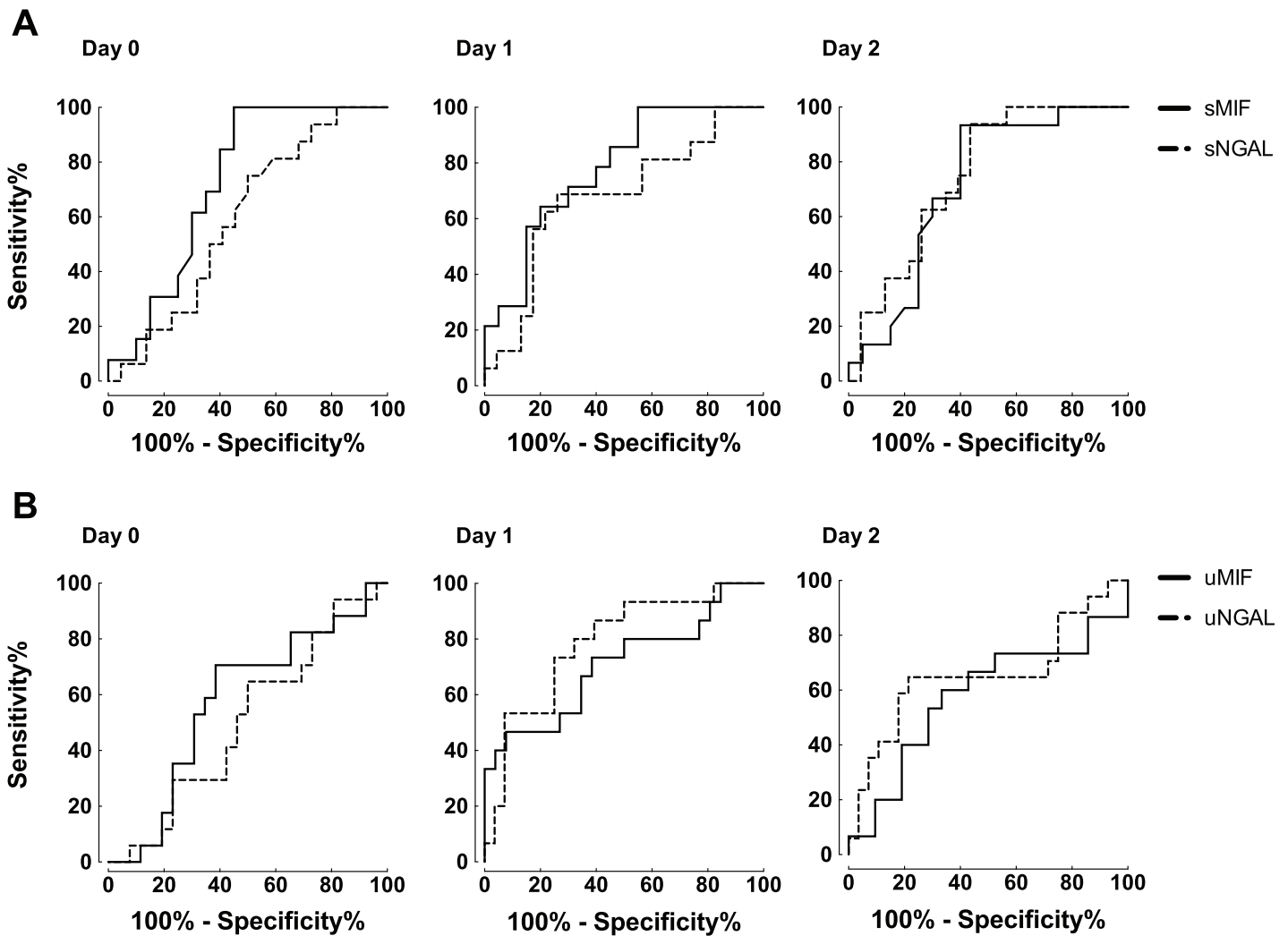
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development of severe AKI after OLT. After OLT, MIF concentrations in serum and urine were greater in patients who developed severe AKI than in those with normal postoperative kidney function. Macrophage migration inhibitory factor had equal power to predict severe AKI after OLT as NGAL, which is an established biomarker for AKI.

Clinical studies showed that NGAL was a good predictor of AKI after cardiac surgery [23], after out of hospital cardiac arrest [24], and in sepsis [25]. In patients undergoing OLT, two studies demonstrated that systemic NGAL was an early predictor of postoperative AKI [4,14]. Niemann et al. reported that a single measurement of plasma NGAL two hours after reperfusion during OLT predicted AKI in patients with a baseline sCr below 1.5 mg/dl [4], while Portal et al. stated that a single measurement of plasma NGAL within 24 hours after OLT predicted AKI with high accuracy [14]. Urinary NGAL was also proposed as an early marker for AKI after OLT. Two studies demonstrated that urinary NGAL concentrations measured within 24 hours after OLT could predict AKI after OLT [14,16], while Wagener et al. reported that urinary NGAL was able to predict AKI at 3 and 18 hours after OLT [15]. Our study confirmed the predictive value of urinary NGAL on day 1 after OLT, while serum NGAL only confirmed diagnosis of severe AKI on day 2 after OLT. Neither serum nor urinary NGAL had a predictive value for severe AKI at the end of surgery. These differences between our findings and previous studies might partially be due to the different timing of sample collection. In addition, the classification of AKI varied between studies. Previous studies classified AKI according to the Risk, Injury, Failure (RIFLE) criteria or the acute kidney injury network (AKIN) criteria, whereas we used the KDIGO criteria. Furthermore, not all authors differentiated between mild and severe AKI, but compared patients with AKI to those without AKI.

Macrophage migration inhibitory factor is a pro-inflammatory cytokine mediating the inflammatory response [26]. Elevated MIF concentrations lead to leukocyte chemotaxis and activation [27,28], resulting in histopathological damage of organs such as lungs [29] or kidneys [30]. Experimental studies have demonstrated the pathogenic role of MIF in immune-mediated renal injury [31,32], crescentic glomerulonephritis [33], and podocyte injury [34]. In humans, the role of MIF in kidney disease is less well described. Systemic MIF concentrations are elevated in patients with chronic kidney disease [35] and in septic patients with AKI [17]. Recently, we have demonstrated elevated plasma MIF concentrations in patients undergoing





**Fig 3. Receiver operating characteristic (ROC) curves of MIF and NGAL for predicting severe AKI after OLT.** (A) ROC curves of serum MIF (sMIF, solid line) and serum NGAL (sNGAL, dashed line) at day 0, day 1 and day 2 after OLT. (B) ROC curves of urinary MIF (uMIF, solid line) and urinary NGAL (uNGAL, dashed line) at day 0, day 1 and day 2 after OLT.

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OLT and have suggested plasma MIF as a potential predictor for the requirement of renal replacement therapy after OLT [7]. Furthermore, urinary MIF has been proposed as a diagnostic tool in kidney disease. Urinary MIF concentrations were increased in patients with acute pyelonephritis, acute renal rejection, and proliferative glomerulonephritis, and correlated with renal MIF expression and the degree of renal injury [18–20]. Hong et al. suggested urinary MIF as a biomarker for acute pyelonephritis [19], whereas Brown et al. proposed that urine concentrations of MIF could be used to differentiate between acute transplant rejection and cyclosporine nephrotoxicity in renal transplant patients [20]. In the current study, we confirmed that serum MIF has predictive value for the development of severe AKI after OLT. In addition, our results suggest that urinary MIF might have a predictive value for severe AKI in the setting of OLT.

Our decision to focus on prediction of severe AKI was based on data from a previous investigation. Waikar et al. proposed that using small changes in creatinine to define AKI would

**Table 4. Area under the ROC curve for development of any stage of AKI.**

	Serum MIF	Serum NGAL	Urine MIF	Urine NGAL
Day 0				
AUC	0.59	0.64	0.65	0.55
95% CI	0.36–0.83	0.44–0.85	0.51–0.78	0.38–0.66
P Value	0.48	0.23	0.05	0.62
Day 1				
AUC	0.81	0.82	0.73	0.59
95% CI	0.61–1	0.63–0.99	0.51–0.99	0.37–0.82
P Value	0.02	0.008	0.06	0.4
Day 2				
AUC	0.86	0.88	0.73	0.7
95% CI	0.73–0.99	0.77–1	0.45–1	0.57–0.93
P Value	0.006	0.002	0.12	0.06

Abbreviations: AKI, acute kidney injury; AUC, area under the curve; CI, confidence intervals; MIF, macrophage migration inhibitory factor; NGAL, neutrophil gelatinase-associated lipocalin-2.

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lead to a high sensitivity but low specificity, and would significantly reduce the reliability of the novel biomarker [36]. Therefore, the investigators suggested using stages 2 and 3 of AKI to assess the predictive performance of novel biomarkers. In order to validate the performance of MIF as a novel biomarker for severe AKI, we compared its power to predict severe AKI to that of the well-established biomarker NGAL. Our results indicate that serum MIF has a fair predictive value for severe AKI after OLT on day 0, whereas the first time point at which urinary MIF and urinary NGAL had a fair predictive performance was on day 1. Due to the small number of patients these results can only be viewed as hypothesis-generating. We also assessed the predictive performance of MIF and NGAL for the development of AKI in general, i.e. stage 1, 2 or 3. The power of MIF and NGAL to predict any stage of AKI at the end of OLT was poor. At day 1 after OLT, serum MIF and serum NGAL had good predictive performance for AKI, as indicated by an AUC > 0.8 for both parameters. Urinary MIF and urinary NGAL did not predict the development of AKI at the end of OLT and on day 1 after OLT. Taken together, these results suggest that both serum MIF and serum NGAL have similar power to predict AKI after OLT. In contrast, urinary MIF and urinary NGAL only predict severe AKI after OLT. Furthermore, based on our results we speculate that serum MIF might be an earlier indicator for severe AKI after OLT than serum NGAL and urinary NGAL.

Another previously un-described finding of this study is the elevation of urine MIF concentrations in patients undergoing OLT. Urine MIF concentrations peak in all patients at the end of OLT, decrease on day 1, and return to baseline values on day 2. This finding might partially be explained by the renal clearance of systemic MIF, which was markedly elevated at the end of OLT. However, patients who developed severe AKI had greater urine MIF concentrations on day 1 after OLT than patients with normal kidney function. Previous studies have demonstrated that renal MIF is constitutively expressed in the normal kidneys and is upregulated in inflammatory kidney disease [18,37]. The upregulation of MIF correlated significantly with the concentration of urinary MIF in patients with glomerulonephritis, pyelonephritis and renal allograft rejection, while systemic MIF concentrations were not increased in these patients. The authors concluded that urinary MIF concentrations increase due to MIF production and secretion by the injured kidney, and proposed that urinary MIF might be a more specific parameter for kidney injury than systemic MIF [18–20]. In particular, Hong et al. suggested that urinary MIF, but not serum

MIF, was an indicator for AKI in patients with acute pyelonephritis [19]. In contrast, we report elevated concentrations of serum and urinary MIF in patients developing AKI after OLT. Serum MIF predicted AKI after OLT earlier than urinary MIF. An explanation for these variable results might be different pathomechanisms in pyelonephritis-induced AKI and AKI after OLT. In pyelonephritis, AKI develops mainly due to a local infection ascending from the urogenital tract towards the kidneys, and thus is classified as renal or postrenal AKI. After OLT, the development of AKI is primarily based on prerenal causes including hypovolemia and hypotension, which result in renal hypoperfusion and ischemia.

Of note, there are limitations for the use of MIF as a biomarker for AKI, which are similar to those of using NGAL as a biomarker. A variety of human cells, including immune cells and hepatocytes, express MIF and NGAL [26,38]. Hence, any injury to those cells would increase systemic MIF and NGAL concentrations. In addition, MIF and NGAL are mediators of the innate immune response [39,40], and systemic concentrations of MIF and NGAL increase in patients with inflammatory diseases [41,42]. Therefore, the release of MIF and NGAL under various circumstances not primarily related to kidney injury could reduce the specificity of these proteins as biomarkers for early prediction of AKI. Furthermore, an overall limitation of this study is the small number of patients enrolled. In addition, the preoperative sCr of all included patients was below 1.5 mg/dl, suggesting absence of preexisting renal dysfunction. Thus, the question whether MIF can predict AKI in patients with preoperative renal dysfunction remains to be answered.

In conclusion, the results of this study suggest that serum MIF can predict AKI at an early postoperative stage after OLT. In the setting of OLT, MIF showed similar potency in predicting severe AKI after OLT as NGAL, and therefore might be useful as a novel biomarker for severe AKI.

## Supporting information

**S1 Fig. Perioperative serum creatinine concentrations at 4 different time points: baseline (BL; under anesthesia before skin incision), day 0 (at the end of surgery), day 1 (24 hours after graft reperfusion on day 1 after OLT), and on day 2 (48 hours after reperfusion day 2 after OLT).** White bars indicate values of patients with no AKI or stage 1 AKI, gray bars represent values of patients who developed stage 2 or 3 AKI after undergoing OLT. P values indicate significant differences between groups. (EPS)

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