SHOCK, Vol. 58, No. 1, pp. 14–19, 2022

OPEN

SUBCLINICAL KIDNEY INJURY IS CAUSED BY A MODERATE SINGLE INFLAMMATORY EVENT

Matthias Wolfgang Heinzl,*† Michael Resl,*† Carmen Klammer,*† Paul Fellinger,§ Lukas Schinagl,* Florian Obendorf,* Roland Feldbauer,* Johannes Pohlhammer,* Thomas Wagner,* Margot Egger,‡ Benjamin Dieplinger,‡ and Martin Clodi*†

**Department of Internal Medicine, Konventhospital Barmherzige Brueder Linz (St. John of God Hospital Linz), Linz, Austria; †Institute for Cardiovascular and Metabolic Research, Johannes Kepler Universität Linz, Linz, Austria; ‡Department of Laboratory Medicine, Konventhospital Barmherzige Brueder Linz (St. John of God Hospital Linz), Linz, Austria; and §Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria*

Received 22 Feb 2022; first review completed 14 Mar 2022; accepted in final form 14 Apr 2022

ABSTRACT—**Background:** Current means of diagnosis of acute kidney injury (AKI) based on serum creatinine have poor sensitivity and may miss possible therapeutic windows in subclinical kidney injury, especially in septic AKI. Kidney injury molecule-1 (KIM-1) may be a valuable biomarker to improve diagnostic algorithms for AKI. The understanding of septic AKI is still insufficient, and knowledge about KIM-1 kinetics in inflammation is scarce. The aim of this study was to investigate the possible effect of lipopolysaccharide (LPS) on KIM-1 as a marker of structural kidney injury in healthy volunteers. **Methods:** A single-blinded, placebo-controlled cross-over study using the human endotoxin model (LPS administration) was performed in 10 healthy men. Kidney injury molecule-1 and serum creatinine were measured repetitively for 48 hours. **Results:** We observed a significant elevation of serum KIM-1 levels after the administration of LPS ($P < 0.001$). Furthermore, LPS caused a significant elevation of serum creatinine at an early time point ($P = 0.013$) as compared with placebo. **Conclusion:** Even a relatively small inflammatory stimulus is sufficient to cause subclinical structural kidney injury with elevated KIM-1 and serum creatinine in healthy volunteers. This outlines the insufficiency of the current diagnostic approach regarding AKI and the urgency to develop novel diagnostic algorithms including markers of kidney injury. **Clinical Trial Registration:** www.clinicaltrials.gov. Unique identifier: NCT03392701 (August 1, 2018)

KEYWORDS—Kidney injury molecule 1, acute kidney injury, inflammation, lipopolysaccharide, human endotoxin model

ABBREVIATIONS—AUC—area under the curve; CKD—chronic kidney disease; CRP—C-reactive protein; ICU—intensive care unit; IL-6—interleukin-6; KIM-1—Kidney injury molecule-1; LPS—Lipopolysaccharide; NPX—normalized protein expression; RM-ANOVA—repeated-measures analysis of variance; sKIM-1—serum Kidney injury molecule-1; uKIM-1—urinary Kidney injury molecule-1

INTRODUCTION

Acute kidney injury (AKI) and renal failure are major and common complications in human sepsis with great morbidity and mortality (1,2). Even patients with more moderate infections not meeting the criteria of sepsis show increased rates of AKI (3). Although the pathophysiology of sepsis-induced renal failure is yet to be fully understood in detail, it has become evident that, unlike previously thought, hypovolemia and renal hypoperfusion are not the primary causes of septic AKI. Multiple underlying factors seem to play crucial roles, including microthromboses in the glomerula, microvascular dysfunction, dysregulation of the intracellular energy homeostasis and subsequent tubule necrosis, infiltration of immune cells, dysfunction of the endothelium, and changes in hemodynamics (1,3).

According to the Kidney Disease: Improving Global Outcomes guidelines (4), AKI is currently diagnosed based on changes in the excretory kidney function as measured by serum creatinine and urine output. However, deterioration of these parameters does not seem to be sensitive enough and, importantly, seems to enable the diagnosis only at a rather late stage of AKI, thus lagging behind early or "subclinical" stages and potentially missing a therapeutic window.

Because it has been shown that biomarker-guided early intervention to prevent AKI may be a promising approach in some settings such as major surgery (5), the identification of reliable, sensitive, and early biomarkers of AKI with potential prognostic value is of considerable importance. There has thus been great interest in the development and validation of novel parameters reflecting kidney injury rather than kidney function to enable clinicians to counteract early, mitigate renal damage, and improve outcome (6,7).

Kidney injury molecule-1

Kidney injury molecule-1 (KIM-1) is a transmembrane molecule mostly expressed in the proximal tubule of the kidneys under acute and chronic kidney damage (8,9). Whereas KIM-1 is hardly detectable in healthy kidneys, it is extensively increased when kidney damage occurs and seems to confer the potential of phagocytosis of apoptotic cells and cell debris to endothelial cells in the renal tubules after injury (8). Furthermore, these phagocytotic properties of KIM-1 result in a pronounced anti-inflammatory effect via downregulation of NF-κB (10).

Address reprint requests to Martin Clodi, MD, Department of Internal Medicine, Konventhospital Barmherzige Brueder Linz (St. John of God Hospital Linz), Seilerstaette 2, 4021 Linz, Austria. E-mail: martin.clodi@bblinz.at.

This study was supported by funds from the Oesterreichische Nationalbank (Oesterreichische Nationalbank, Anniversary Fund, project number: 16415).

The authors acknowledge the support of the NIH Clinical Center in providing the endotoxin used in this study.

The authors report no conflict of interests.

DOI: 10.1097/SHK.0000000000001942

Kidney injury molecule-1, which can be measured in serum (serum kidney injury molecule-1 [sKIM-1]) and urine (urine kidney injury molecule-1 [uKIM-1]), seems to be a potential early, sensitive, and easily applicable marker of acute structural kidney damage, as shown in rodent models (11) and in clinical data for AKI (6,12), and may be of even increased value in combination with interleukin (IL)-18 (13). Even in subcollectives of chronic kidney disease, KIM-1 seems to be associated with long-term outcome, such as progression of diabetic nephropathy in type 1 diabetes (9), glomerulonephritis, or vasculitis (14). This could indicate a potential role as a predictive marker in chronic kidney disease patients with predominant tubulointerstitial damage or inflammation (14).

In pooled analysis from multiple studies, uKIM-1 showed good sensitivity and specificity regarding AKI with however large inconsistency depending on the clinical setting and, importantly, the time of measurement after possible kidney damaging events such as cardiac surgery (12). This outlines the need to develop algorithms for the timing of the analysis of markers of kidney damage or injury such as KIM-1 for the everyday clinical setting in patients at risk for AKI, such as postoperative, septic, or intensive care unit (ICU) patients.

It has been shown that sKIM-1 is elevated in septic patients developing AKI at 6 hours after ICU admission, preceding a rise in serum creatinine by almost 24 hours (6). Urine kidney injury molecule-1 was furthermore significantly elevated at 24 hours and 48 hours after ICU admission in septic AKI patients with predictive value regarding mortality (6).

To analyze the possible utility of KIM-1 and its predictive role in AKI, knowledge of its kinetics is essential. However, detailed kinetics of uKIM-1 and sKIM-1 after inflammatory events is still insufficient.

Human endotoxin model

Administration of lipopolysaccharide (LPS) from gram-negative bacteria reliably causes a febrile systemic inflammatory response in healthy individuals. This human endotoxin model is a safe and the most widely accepted and standardized model to study the pathophysiology of human host response to infection and has been particularly useful to determine the kinetics of various parameters in human inflammation and sepsis (15–24).

In summary, there is room for amelioration to the current approach to screen for AKI, especially at early stages. The inclusion of markers of kidney injury, such as KIM-1, may provide valuable benefits and allow more sensitive and earlier diagnosis with potentially significant impact on patient care. The aim of this study was to determine the possible effect of a single inflammatory stimulus on KIM-1 as a marker of kidney injury in healthy individuals using the human endotoxin model to test the hypotheses that KIM-1 may be elevated in inflammation.

METHODS

This study was reviewed and approved by the Institutional Review Board of the St. John of God Hospital Linz and the ethics committee of the Medical University of Vienna. Informed consent was obtained in writing and orally from each subject before enrolment in the study. All methods were performed in accordance with the relevant guidelines and regulations.

Protocol

The human endotoxin model was performed as described previously (18,22,24) in 10 healthy male volunteers. In brief, placebo (0.9% NaCl) and LPS at a dose of 2 ng/kg (National Reference Bacterial Endotoxin; lot no. 94332B1, generously provided by the Investigational Drug Management at the National Institutes of Health, Bethesda, Maryland) were administered intravenously as a bolus to 10 healthy nonsmoking male LPS-naive volunteers in a single-blinded cross-over design on 2 different study days (6 volunteers received placebo on the first study day and LPS on the second day, 4 volunteers received LPS on the first and placebo on the second day with a washout phase of at least 2 weeks).

Study days were started at 8:00 AM after an overnight fast. Subjects received 300 mL of 0.9% saline for 90 minutes at the beginning of the study day and were allowed to drink 1.5 L of nonsparkling mineral water. Vital sign monitoring and repetitive blood sampling were performed for 6 hours; participants rested in a supine position during this period. Blood samples were also taken 24 hours and 48 hours after infusion at a fasting state.

Laboratory measurements and statistical analysis

Blood was taken using VACUETTE polyethylene terephthalate glycol blood collection tubes (Greiner Bio-One). Interleukin 6, C-reactive protein (CRP), and serum creatinine were determined within 2 hours of blood collection in all study participants in lithium-heparin plasma (18).

Analysis of sKIM-1 was performed using the proximity extension assay technology. For this purpose, ethylenediaminetetraacetic acid-plasma samples (frozen at −80°C) were sent to Olink Proteomics in Uppsala (Sweden). Proximity extension assay is an immunoassay based on a pair of specific antibodies to a predefined antigen. These antibodies are bound to DNA oligonucleotides with affinity for each other. Consequently, the oligonucleotides are hybridized upon target binding, thus forming a new polymerase chain reaction target sequence with subsequent extension and amplification by a DNA polymerase and quantification by quantitative real-time polymerase chain reaction (25). Biomarker concentrations are given in normalized protein expression (NPX) units, which is an arbitrary unit on a log2 scale, meaning an increase by one NPX unit reflects a two-fold increase in biomarker concentration (26). Areas under the curve (AUCs) were calculated using the trapezoidal method with GraphPad Prism (GraphPad Software, San Diego, CA).

Statistical analysis was performed using IBM SPSS Statistics 26 and 27 and GraphPad Prism version 9 (GraphPad Software, San Diego, CA). Statistical tests included paired *t* tests, Pearson coefficient of correlation, repeated-measures analysis of variance (RM-ANOVA), and Wilcoxon matched-pairs signed rank test. When sphericity could not be assumed according to Mauchly test of sphericity, the Greenhouse-Geisser correction was used. The primary analysis of this study was RM-ANOVA of KIM-1.

RESULTS

Inflammation after LPS infusion

As described previously (18,22,24), 10 healthy male nonsmoking volunteers (mean age, 24.1 years [SD, 3.7 years]; mean body mass index, 25.2 kg/m^2 [SD, 1.6) kg/m^2]) were included in the study. Interleukin 6 and CRP were significantly elevated after LPS infusion. Although all but one subject experienced chills, myalgia, mild to moderate headache, or other flu-like symptoms, these symptoms were rather short-lived. At 5 hours after LPS infusion, virtually all symptoms had abated; most of them had already subsided at 3 hours after infusion (18).

KIM-1 in inflammation

During the first 3 hours after the infusion of LPS and placebo, serum levels of KIM-1 decreased slowly without relevant differences between the 2 study days. After 180 minutes, differences became apparent with serum concentrations of KIM-1 rising at 360 minutes with a maximum at 24 hours after LPS infusion, remaining significantly elevated at 48 hours after LPS, whereas KIM concentrations after infusion of placebo did not rise at all in comparison with baseline. This difference between the 2 study days was statistically significant, as calculated by RM-ANOVA ($F = 8.336$, $P < 0.001$, Fig. 1). In secondary analysis, direct comparison at the same time point using paired *t* tests revealed significant differences at 24 hours ($P = 0.011$) and 48 hours ($P = 0.016$) but not at 360 minutes ($P = 0.249$). There was no significant difference at baseline between the 2 study days ($P = 0.225$).

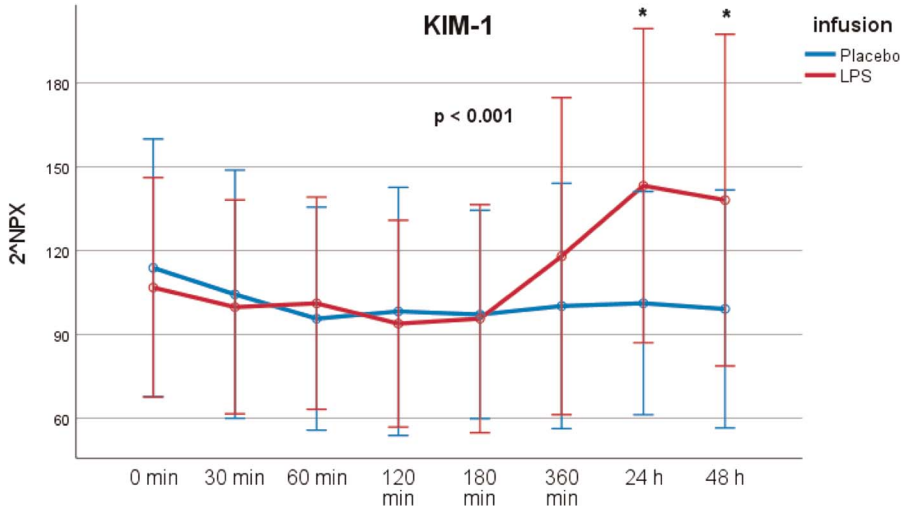

Fig. 1. **Serum KIM-1 levels after administration of placebo and LPS.** Statistical analysis using RM-ANOVA revealed a significant difference ($P < 0.001$) with sKIM-1 concentrations rising from 6 hours after LPS administration. In secondary analysis, paired *t* tests revealed significant differences at 24 hours ($P = 0.011$) and 48 hours ($P = 0.016$). Of note, the NPX format is an arbitrary log2 unit; thus, the depicted values do not reflect actual concentrations. For better graphical depiction, the log2-logarithmic NPX values were transformed into linear scale by calculating $2^{(\text{respective NPX value})}$. Statistical analysis was performed using the original NPX values.

The AUC of KIM-1 over the observation period of 48 hours was significantly higher in the LPS group compared with placebo (median difference, 64,789 $NPX^2 \times$ min; $P = 0.0039$; Fig. 2).

Serum creatinine after LPS

After placebo infusion, serum creatinine decreased from baseline levels (fasting state) throughout the study day and were similar to baseline at 24 hours and 48 hours (also in a fasting state). Additionally to the significant effect on sKIM-1 levels by the bolus administration of LPS, analysis also revealed a significant increase in serum creatinine levels after LPS administration compared with placebo ($P = 0.013$ as calculated with RM-ANOVA; Fig. 3). In secondary analysis, direct comparison at respective time points using paired *t* test showed significant differences at 60 minutes ($P = 0.024$) and 120 minutes ($P = 0.013$) with no significant difference at baseline ($P = 0.282$).

The AUC of serum creatinine over 48 hours was not significantly different between the LPS and the placebo group. However, the AUC was significantly increased after LPS injection when only analyzing the first 6 hours after LPS injection (median difference, 10.40 mg/dL $\times$ min; $P = 0.0273$; Fig. 4).

Correlation between KIM-1, inflammation, and serum creatinine

Calculating Pearson correlation coefficient revealed that KIM-1 concentrations did not correlate with IL-6 or CRP as markers of inflammation at any time point, nor was there a correlation between maximum levels of serum creatinine and KIM-1 levels (selected time points shown in Table 1). Furthermore, there was no correlation between the maximum levels of serum creatinine and IL-6 or CRP at any time point.

DISCUSSION

Sepsis is the most common cause of AKI in critically ill patients (3). However, even less critical conditions such as nonsevere community-acquired pneumonia frequently cause AKI, which is an important prognostic parameter and a major clinical issue in these patients (27).

Serum creatinine after LPS

In our study, we show that serum creatinine levels are significantly increased after bolus injection of bacterial endotoxin (LPS 2 ng/kg) in comparison with placebo. This increase is small and does not meet the current Kidney Disease: Improving Global

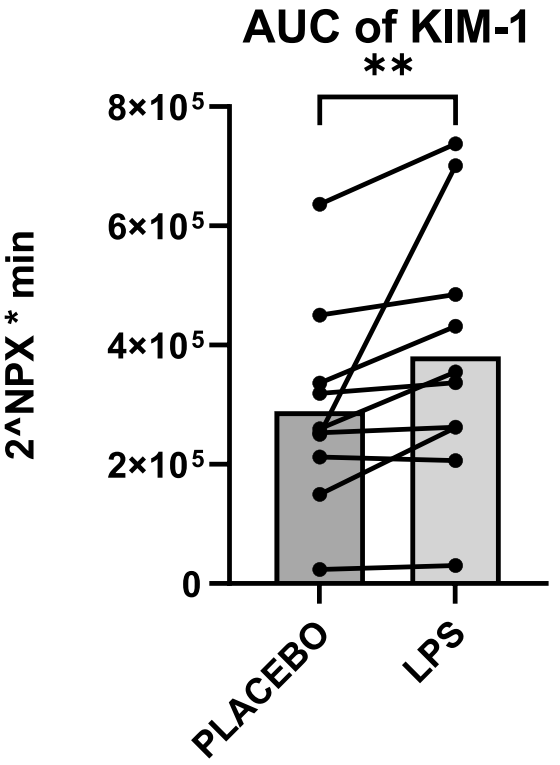

Fig. 2. **Area under the curve of serum KIM-1 levels after administration of LPS and placebo.** Statistical analysis using AUCs calculation with the trapezoidal method over the observation period of 48 hours showed significantly higher AUC for serum KIM-1 after LPS compared with placebo (median difference, 64,789 $NPX^2 \times$ min; $^{**}P = 0.0039$).

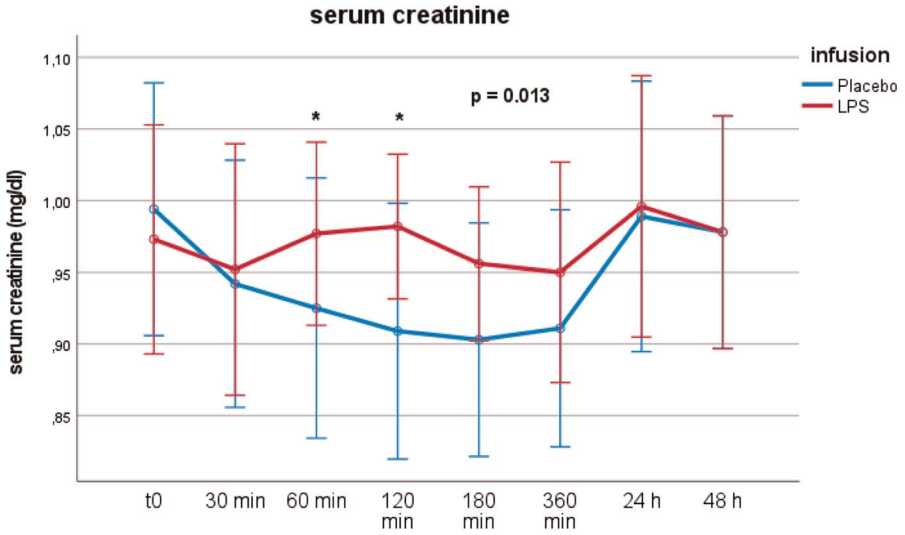

FIG. 3. **Serum creatinine concentrations after administration of placebo and LPS.** Statistical analysis using RM-ANOVA revealed a significant difference (P = 0.013) over 48 hours. In secondary analysis, serum creatinine concentrations showed significantly elevated at 60 minutes (*P = 0.024) and 120 minutes (*P = 0.013) in comparison with placebo using paired t test. There was no significant difference at baseline (P = 0.282). The decrease of serum creatinine after placebo infusion may be due to the fasting state of subjects at the beginning of the study day.

Outcomes criteria for the diagnosis of AKI (4) and would probably not be noted in daily patient care, especially because this effect is blurred by a decrease of serum creatinine from higher levels at baseline (fasting state) after placebo infusion. Nevertheless, it is all the more remarkable to observe such a marked effect induced by a single moderate inflammatory bolus stimulus in young and healthy individuals. Although all but one subject in the study experienced symptoms of varying degree and markers of inflammation such as IL-6 were markedly elevated, all symptoms were rather short-lived, and the severity of the inflammatory stimulus exerted in the study is certainly much less pronounced than that of actual infections in patients requiring hospitalization or even ICU care.

The timing of serum creatinine elevation after LPS is also remarkable: in patients with septic AKI, serum creatinine is usually increased later than 6 hours after ICU admission (6). In our study, serum creatinine is already increased at a much earlier time point, from 60 minutes to 360 minutes after LPS injection compared with placebo (Fig. 3). The fact that this early subclinical but statistically significant elevation of serum creatinine after LPS could be observed in our study but not in clinical studies including septic patients at the ICU may be attributable to the experimental crossover design of our study, which allows to observe much smaller changes in biomarker kinetics in good temporal resolution than clinically relevant effects that can be observed in daily routine patient care.

KIM-1 in inflammation

Importantly, this increase in serum creatinine was accompanied by a significant increase in sKIM-1 (Fig. 1), a protein mainly expressed in the proximal tubule of the kidneys after kidney damage (8,9) with important properties in renal repair and epithelial cell restoration (28). This suggests that the moderate inflammatory stimulus not only causes transient functional renal impairment, as suggested by the rise in serum creatinine, but also seems to cause structural kidney injury. This study is, to the best of our knowledge, the first to describe KIM-1 kinetics after a defined inflammatory stimulus.

Although KIM-1 has been shown to be elevated in various studies of AKI (1,6,13,29), KIM-1 is currently not being used as a diagnostic biomarker to guide treatment or clinical decision making, and its potential value for patient care is still being discussed.

Knowledge of the elevation of KIM-1 and its kinetics in inflammation may also add to the complex pathophysiological picture of septic AKI. Traditionally, AKI in sepsis used to be explained primarily by renal hypoperfusion. In recent years, however, it has become more and more evident that the pathophysiology behind septic AKI is much more complex with multiple different factors involved including impaired microcirculation and microthromboses, direct effects of infectious toxins, and deleterious effects of immune

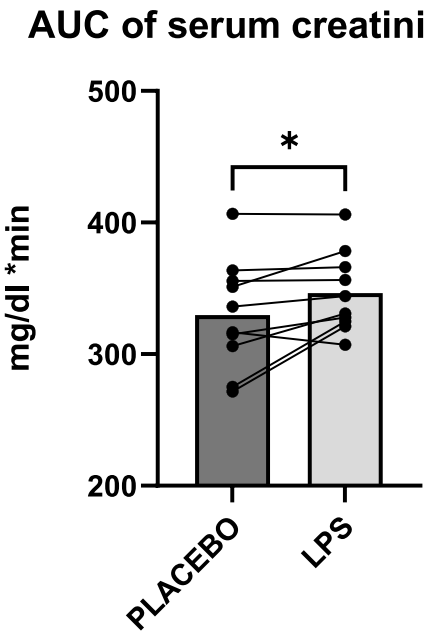

FIG. 4. **Area under the curve of serum creatinine after administration of LPS and placebo.** Statistical analysis using AUCs calculation with the trapezoidal method over 6 hours after infusion showed significantly higher AUC for serum creatinine after LPS compared with placebo (median difference, 10.40 mg/dL × min; *P = 0.0273).

Table 1. **Correlations between levels of sKIM-1 and markers of inflammation as well as serum creatinine**

		Serum creatinine — maximum after LPS	IL-6 at baseline	Peak IL-6 (180 min after LPS)	Peak CRP (24 h after LPS)
KIM-1 at baseline	Pearson coefficient of correlation	–0.358	0.136	0.269	0.301
	P	0.310	0.709	0.453	0.399
KIM-1 — 24 h after LPS	Pearson coefficient of correlation	–0.340	0.222	0.397	0.435
	P	0.337	0.538	0.256	0.209
KIM-1 — 48 h after LPS	Pearson coefficient of correlation	–0.305	0.182	0.341	0.411
	P	0.391	0.615	0.334	0.239

There was no significant correlation between sKIM-1 levels and CRP or IL-6 as markers of inflammation at any time point. Furthermore, there was no correlation between KIM-1 levels at any time point and the maximum concentration of serum creatinine.

mediators and immune cells (1,30). Although the pathophysiology of septic AKI is currently still being debated and most of the data derive from animal models, it is quite ramarkable that the rather moderate inflammatory stimulus used in our study is sufficient to induce signs of functional and structural kidney damage.

Limitations

There are several limitations to our study. Most importantly, we did not measure urine output, nor can we provide urinary biomarkers. Regarding the fluid status of the subjects, everyone drank 1.5 L of nonsparkling mineral water on each of the study days without difference between the days of placebo and LPS administration. Thus, we do not believe that the elevation of serum creatinine is a mere reflection of exsiccation, although alterations in the fluid status between the intracorporal compartments and renal blood flow may be involved in creatinine changes. However, in synopsis, with KIM-1 as a marker of structural renal damage also being elevated, we believe that the elevation of serum creatinine reflects an actual transient impairment in excretory kidney function.

Impact

Although it is known that the duration and severity of AKI is associated with outcome (31), current strategies for early detection of patients at high risk for AKI and timely prophylactic measures for prevention or early treatment of AKI are still unsatisfactory. However, it has been shown that biomarker-guided early implementation of nephroprotective measures including the optimization of fluid status and early discontinuation of nephrotoxic substances reduced moderate and severe AKI incidence and shortened ICU stays after major noncardiac surgery (5). Whether the benefits of such early nephroprotective measures also extend to subclinical stages of septic AKI is yet to be determined.

With KIM-1 and serum creatinine being elevated, we conclude that a comparatively small experimental inflammatory stimulus (bolus injection of 2 ng/kg endotoxin) is sufficient to cause structural kidney damage and a transient functional renal impairment. This is of importance because the current criteria for diagnosing AKI are insufficient to detect such subclinical kidney injury.

Our data thus underscore the necessity for improved diagnostic tools to identify patients at high risk for AKI and patients with subclinical AKI (1) to establish improved therapeutic algorithms to reduce the burden of AKI in sepsis, hoping to reduce the need for renal replacement therapy in ICUs and improve patient outcome in sepsis. Given the effects of a single LPS administration in healthy volunteers on the kidney, septic AKI may still be largely underestimated in daily clinical routine care.

CONCLUSION

In summary, this study is the first to show that KIM-1 is elevated in artificial human inflammation and indicates that subclinical kidney injury occurs after a single inflammatory stimulus (LPS administration in healthy volunteers) with both functional renal impairment, reflected by a rise of serum creatinine, and structural kidney damage, reflected by sKIM-1 elevation.

REFERENCES

1. Bellomo R, Kellum JA, Ronco C, Wald R, Martensson J, Maiden M, Bagshaw SM, Glassford NJ, Lankadeva Y, Vaara ST, et al.: Acute kidney injury in sepsis. *Intensive Care Med* 43:816–828, 2017.
2. Bagshaw SM, George C, Bellomo R, ANZICS Database Management Committee: Changes in the incidence and outcome for early acute kidney injury in a cohort of Australian intensive care units. *Crit Care* 11:R68, 2007.
3. Zarjou A, Agarwal A: Sepsis and acute kidney injury. *J Am Soc Nephrol* 22: 999–1006, 2011.
4. Khwaja A: KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 120:c179–c184, 2012.
5. Gocze I, Jauch D, Gotz M, Kennedy P, Jung B, Zeman F, Gnewuch C, Graf BM, Gnann W, Banas B, et al.: Biomarker-guided intervention to prevent acute kidney injury after major surgery: the prospective randomized BigpAK study. *Ann Surg* 267:1013–1020, 2018.
6. Tu Y, Wang H, Sun R, Ni Y, Ma L, Xv F, Hu X, Jiang L, Wu A, Chen X, et al.: Urinary netrin-1 and KIM-1 as early biomarkers for septic acute kidney injury. *Ren Fail* 36:1559–1563, 2014.
7. Schrezenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM: Biomarkers in acute kidney injury — pathophysiological basis and clinical performance. *Acta Physiol (Oxf)* 219:554–572, 2017.
8. Ichimura T, Asseldonk EJ, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV: Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *J Clin Invest* 118:1657–1668, 2008.
9. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, et al.: Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *J Am Soc Nephrol* 25:2177–2186, 2014.
10. Yang L, Brooks CR, Xiao S, Sabbisetti V, Yeung MY, Hsiao LL, Ichimura T, Kuchroo V, Bonventre JV: KIM-1–mediated phagocytosis reduces acute injury to the kidney. *J Clin Invest* 125:1620–1636, 2015.
11. Vaidya VS, Ramirez V, Ichimura T, Bobadilla NA, Bonventre JV: Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. *Am J Physiol Renal Physiol* 290:F517–F529, 2006.
12. Shao X, Tian L, Xu W, Zhang Z, Wang C, Qi C, Ni Z, Mou S: Diagnostic value of urinary kidney injury molecule 1 for acute kidney injury: a meta-analysis. *PLoS One* 9:e84131, 2014.
13. Arthur JM, Hill EG, Alge JL, Lewis EC, Neely BA, Janech MG, Tumlin JA, Chawla LS, Shaw AD, SAKInet Investigators: Evaluation of 32 urine biomarkers to predict the progression of acute kidney injury after cardiac surgery. *Kidney Int* 85:431–438, 2014.
14. Seibert FS, Sitz M, Passfall J, Haesner M, Laschinski P, Buhl M, Bauer F, Babel N, Pagonas N, Westhoff TH: Prognostic value of urinary calprotectin, NGAL and KIM-1 in chronic kidney disease. *Kidney Blood Press Res* 43:1255–1262, 2018.
15. Fong YM, Marano MA, Moldawer LL, Wei H, Calvano SE, Kenney JS, Allison AC, Cerami A, Shires GT, Lowry SF: The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. *J Clin Invest* 85:1896–1904, 1990.

16. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM, Wilmore DW: Detection of circulating tumor necrosis factor after endotoxin administration. *N Engl J Med* 318:1481–1486, 1988.
17. Vila G, Riedl M, Resl M, van der Lely AJ, Hofland LJ, Clodi M, Luger A: Systemic administration of oxytocin reduces basal and lipopolysaccharide-induced ghrelin levels in healthy men. *J Endocrinol* 203:175–179, 2009.
18. Heinzl MW, Resl M, Klammer C, Egger M, Dieplinger B, Clodi M: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is not induced in artificial human inflammation and is not correlated with inflammatory response. *Infect Immun* 88: e00842–e00819, 2020.
19. Vila G, Resl M, Stelzeneder D, Struck J, Maier C, Riedl M, Hulsmann M, Pacher R, Luger A, Clodi M: Plasma NT-proBNP increases in response to LPS administration in healthy men. *J Appl Physiol* 105(6):1741–1745, 1985.
20. Clodi M, Vila G, Geyeregger R, Riedl M, Stulnig TM, Struck J, Luger TA, Luger A: Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *Am J Physiol Endocrinol Metab* 295:E686–E691, 2008.
21. Vila G, Maier C, Riedl M, Nowotny P, Ludvik B, Luger A, Clodi M: Bacterial endotoxin induces biphasic changes in plasma ghrelin in healthy humans. *J Clin Endocrinol Metab* 92:3930–3934, 2007.
22. Millischer V, Heinzl M, Faka A, Resl M, Trepci A, Klammer C, Egger M, Dieplinger B, Clodi M, Schwieler L: Intravenous administration of LPS activates the kynurenine pathway in healthy male human subjects: a prospective placebo-controlled cross-over trial. *J Neuroinflammation* 18:158, 2021.
23. Andreasen AS, Krabbe KS, Krogh-Madsen R, Taudorf S, Pedersen BK, Moller K: Human endotoxemia as a model of systemic inflammation. *Curr Med Chem* 15: 1697–1705, 2008.
24. Resl M, Heinzl MW, Klammer C, Egger M, Feldbauer R, Pohlhammer J, Dieplinger B, Clodi M: Storm of cardiovascular markers after LPS administration in human volunteers. *J Cardiovasc Transl Res* 14:941–947, 2021.
25. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, et al.: Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 9:e95192, 2014.
26. Shen Q, Bjorkesten J, Galli J, Ekman D, Broberg J, Nordberg N, Tillander A, Kamali-Moghaddam M, Tybring G, Landegren U: Strong impact on plasma protein profiles by precentrifugation delay but not by repeated freeze-thaw cycles, as analyzed using multiplex proximity extension assays. *Clin Chem Lab Med* 56:582–594, 2018.
27. Murugan R, Karajala-Subramanyam V, Lee M, Yende S, Kong L, Carter M, Angus DC, Kellum JA, Genetic and Inflammatory Markers of Sepsis (GenIMS) Investigators: Acute kidney injury in non-severe pneumonia is associated with an increased immune response and lower survival. *Kidney Int* 77:527–535, 2010.
28. Yin C, Wang N: Kidney injury molecule-1 in kidney disease. *Ren Fail* 38: 1567–1573, 2016.
29. Khreba NA, Abdelsalam M, Wahab AM, Sanad M, Elhelaly R, Adel M, El-Kannishy G: Kidney injury molecule 1 (KIM-1) as an early predictor for acute kidney injury in post-cardiopulmonary bypass (CPB) in open heart surgery patients. *Int J Nephrol* 2019:6265307, 2019.
30. Kellum JA, Prowle JR: Paradigms of acute kidney injury in the intensive care setting. *Nat Rev Nephrol* 14:217–230, 2018.
31. Peerapornratana S, Priyanka P, Wang S, Smith A, Singbartl K, Palevsky PM, Chawla LS, Yealy DM, Angus DC, Kellum JA, et al.: Sepsis-associated acute kidney disease. *Kidney Int Rep* 5:839–850, 2020.