Journal of Translational Medicine

RESEARCH Open Access

# Dynamic changes in the real-time glomerular filtration rate and kidney injury markers in different acute kidney injury models

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## Abstract

In this study, we dynamically monitored the glomerular filtration rate and other assessment of renal function and markers of injury in various mice models of acute kidney injury. Male C57BL/6 mice were utilized to establish acute kidney injury models of sepsis, ischemia reperfusion, cisplatin, folic acid, aristolochic acid and antibiotic. In addition to the real time glomerular filtration rate, renal LCN-2 and HAVCR-1 mRNA expression levels, and serum creatinine, urea nitrogen and cystatin c levels were also used to evaluate renal function. In addition, the protein levels of LCN-2 and HAVCR-1 in renal, serum and urine were measured. Our results demonstrated that the changes in biomarkers always lagged the real time glomerular filtration rate during the progression and recovery of renal injury. Cystatin-c can reflect renal injury earlier than other markers, but it remains higher in the recovery stage. Perhaps the glomerular filtration rate does not reflect the greater injury caused by vancomycin plus piperacillin.

**Keywords** Acute kidney injury, Real time glomerular filtration rate, Sepsis, Ischemia reperfusion, Cisplatin, Folic acid, Aristolochic acid, Vancomycin, Piperacillin tazobactam, Nephrotoxicity

## Background

Acute kidney injury (AKI) is associated with prolonged hospital stays and high mortality and affects 30 to 60% of critically ill patients. It is mainly can be caused by sepsis, ischemia–reperfusion, or nephrotoxic drugs [1]. AKI is mostly defined as a decrease in the glomerular filtration rate (GFR) [2]. The current diagnosis is mostly based on an increase in serum creatinine (Scr) concentration and/or a decrease in urine output. However, the serum creatinine concentration and urine output may also be affected by nonrenal-related factors and may not accurately reflect renal injury [3]. Late diagnosis has hindered our understanding of the pathophysiological mechanisms of AKI and the exploration of specific pharmacologic treatments.

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Less is known about the relationship and time course of injury relative to functional changes in the kidney. Thus, there is a critical need to assess the relationship between real-time GFR and standard biomarkers of kidney injury. Schock-Kusch et al. first reported the use of percutaneous monitored real-time glomerular filtration rate technology in rats in 2009 [4]. Currently, this technique has been used in diverse mouse models, such as healthy mice, nephrectomized mice, and animals with nephronophthisis [5]. In this study, we evaluated the real-time glomerular filtration rate in mice with sepsis, ischemia–reperfusion, cisplatin, folic acid, aristolochic acid, vancomycin, piperacillin tazobactam, and vancomycin combined with piperacillin tazobactam associated renal injury.

## Materials and methods

### Animals

Male C57BL/6 mice aged 8 to 10 weeks were acquired from Charles River Laboratories (Beijing). Animals were maintained individually in vivo with 12-h light/dark cycles and access to food and water ad libitum. The animals were allowed to acclimate for 7 to 10 days before the experimental procedures were conducted. Approval was obtained from the institutional animal care use committee before study commencement. Only healthy mice were used for the experiments; the glomerular filtration rate was measured for each mouse before the start of the experiment, and mice with abnormal renal function were excluded.

### Acute kidney injury model

Acute kidney injury models were established as follows:

1. **Acute kidney injury in sepsis**

1.1. Lipopolysaccharide (LPS): 10 mg/kg, single injection, intraperitoneal injection (IP).

1.2. LPS: 2 mg/kg, single injection (IP).

2. **Folic acid, aristolochic acid and cisplatin-associated kidney injury**

2.1. Folic acid: 300 mg/kg, single injection (IP).

2.2. aristolochic acid: 10 mg/kg, daily injections (IP).

2.3. Cisplatin: 30 mg/kg, single injection (IP).

3. **Ischemia–reperfusion acute kidney injury**

3.1. Ischemia–reperfusion: Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg, IP), and the bilateral abdominal hair of the mice was removed with hair clippers to expose the surgical area. Bilateral lateral abdominal incisions were identified to expose the kidneys, and the bilateral renal pedicles were clamped for 30 min using a noninjury-free microvascular clip. The fixture was then removed, and renal ischemia and reperfusion were monitored visually. Finally, surgical sutures were used to close the bilateral abdominal musculature. The body temperature of the mice was maintained at 37 °C throughout the operation, and the body temperature was monitored [6]. After surgery, the mice had free access to food and water.

4. **Antibiotic-associated kidney injury**

4.1. Vancomycin: 150 mg/kg, daily injections, intravenous injections (IV).

4.2. Piperacillin: 1400 mg/kg, daily injections (IP).

4.3. Vancomycin combined with piperacillin: Vancomycin: 150 mg/kg, daily injections (IV); piperacillin: 1400 mg/kg, daily injections (IP).

### GFR measurement in conscious animals

Transcutaneous measurements were performed as previously described [5]. One day before the experiment, the area on the flank was shaved, and a depilation cream was applied to remove the remaining fur to facilitate transdermal readout. We briefly anesthetized the mice with isoflurane before the real-time GFR was measured. An optical device (MediBeacon GmbH, Mannheim, Germany) was attached to the shaved area and held in place with an elastic gauze bandage. Approximately 3–5 min after the mice had regained consciousness, background signals were measured to obtain stable baseline values, and FITC-sinistrin was injected through the tail vein (0.7 mg/kg, FITC-sinistrin was dissolved in PBS at a concentration of 35 mg/ml). The optical device and data were downloaded and analyzed using MB Studio v.22 (MediBeacon GmbH) after removal of the device under isoflurane anesthesia following continuous monitoring for approximately 1 h. In addition, for the sepsis model, FITC-sinistrin was continuously injected into the tail vein every 2 h to monitor the glomerular filtration rate at 2, 4, 6 and 8 h after the model was established. The real-time GFR was calculated from the FITC-sinistrin clearance using a three-compartment model with linear baseline correction. For continuous monitoring for three-compartment model calculation of the baseline values we adopt measure baseline values for the first time. To minimize the influence of other factors on the real-time GFR, the animals were measured at the same time of day under conditions such as the same ambient temperature and light. Due to the non-invasive technique of GFR monitoring, we performed GFR and urine related markers in the same mouse consecutively. For the other assays, we separately set up a group to perform assays after sacrificing mice.

### Biochemical measurements

Serum creatinine levels were assessed using reagent kits (Scr; cat. no. c011-2-1) according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute). Serum urea nitrogen was measured by an automatic biochemical analyzer (IDEEX, Catalyst One*Analyzer, USA). Cystatin C, LCN-2 and HAVCR-1 were assessed using reagent kits (E-EL-M3024, E-EL-M0828, E-EL-M3039) according to the manufacturer's instructions (Elabscience, Wuhan, China.)

### RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT–PCR)

Total RNA was isolated from renal tissues using TRIzol reagent (Invitrogen, San Diego, CA, USA). cDNA was obtained from 1 μg of RNA according to the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Relative levels of the target genes were determined via qPCR using Ultra SYBR Mixture (2x SYBR Green PCR Master Mix, Bimake, no. B21203). The PCR sequences of primers used are as follows: GAPDH (forward: 5'-ACTCCACTCACGGCAAATTC-3', reverse: 5'-TCTCCATGGTGGTGAAGACA-3'); LCN-2 (forward: 5'-GGACCAGGGCTGTCGCTACT', reverse: 5'-GGTGGCCACTTGCACATTGT-3'); and HAVCR-1 (forward: 5'-GTCTGTATTGTTGTCGAGTGGAG-3', reverse: 5'-CGTGTGGGAATCTCTGGTTTAAC'). Transcript expression was calculated using the comparative Ct method and was normalized to that of GAPDH ($2-\Delta\Delta Ct$).

### Immunofluorescent staining

For immunohistochemical staining, primary antibody for LCN-2 (ABS 043-29-02, ThermoFisher) or HAVCR-1 (NBP1-76701, Novus) was applied to sections overnight at 4 °C. Sections were then incubated with the corresponding secondary antibody (Ab150075, Abcam). Finally, sections were covered with DAPI (Sigma-Aldrich) containing anti-fading medium (Invitrogen), and images were captured using a Olympus confocal microscope. Five fields were randomly selected from each section for positive area statistics.

### Statistical analysis

The data are presented as the mean ± SEM. One-way analysis of variance (ANOVA), two-way ANOVA, and three-way ANOVA were used to compare the differences among multiple groups when needed plus Tukey's post hoc test. All the statistical analyses were performed using GraphPad Prism 9.4.1. P values $< 0.05$ were considered to indicate statistical significance.

## Results

### Acute kidney injury in sepsis

In a model of acute kidney injury induced by different doses of LPS, we measured the real-time GFR percutaneously after LPS injection. The results showed that GFR in both low and high dose groups decreased significantly at 2 h after LPS injection, then slowly decreased, and slowly recovered in the following 1, 2, and 3 days. The mild sepsis group returned to the normal level, while the severe sepsis group still maintained a low level of GFR (Fig. 1a). The levels of serum creatinine, urea nitrogen, NGAL and KIM-1 in the kidney and urine were close to the trend of GFR (Fig. 1b, c, g, h), but did not reflect the renal dysfunction in the severe sepsis-induced acute kidney injury group at the stage of renal function recovery. Serum levels of cystatin c, NGAL, and KIM-1 remained at a certain delay (Fig. 1d, e, and f), and all markers lagged behind GFR.

### Folic acid, aristolochic acid and cisplatin associated acute kidney injury

In the animal model of acute kidney injury induced by folic acid, GFR only decreased at 24 h and then gradually returned to normal level (Fig. 2a). Serum NGAL and KIM-1 levels increased earlier than other markers, but renal tissue NGAL/KIM-1 levels and serum cystatin c gradually accumulated. In the animal model of acute kidney injury induced by aristolostolochia acid and cisplatin, GFR gradually decreased, reaching the lowest value on the third day (Fig. 2a), indicating that the kidney injury was gradually aggravated, and this was also reflected by other biomarkers(Fig. 2).

### Ischemia-reperfusion acute kidney injury

In the ischemia−reperfusion model, we found that the GFR and Scr level were significantly different after 8 h of reperfusion, and the GFR decreased to the lowest level at 24 h, the serum levels of NGAL and KIM-1 also increased at 8 h. The BUN level and renal tissue NGAL mRNA level began to increase significantly at 24 h and peaked at 48 h, while the KIM-1 mRNA level began to increase, peaked at 24 h, and then gradually decreased (Fig. 3).

### Antibiotic associated acute kidney injury

In animal models of antibiotic-induced kidney injury, GFR decreased on the first day in vancomycin and on the second day in piperacillin.The levels of serum creatinine, urea nitrogen and cystatin c increased only slightly on day 2 or day 3. There was no significant change in renal NGAL and KIM-1 mRNA levels. In the vancomycin plus piperacillin animal model, the real-time GFR remained unchanged, but serum urea nitrogen and cystatin c levels increased significantly (Fig. 4).

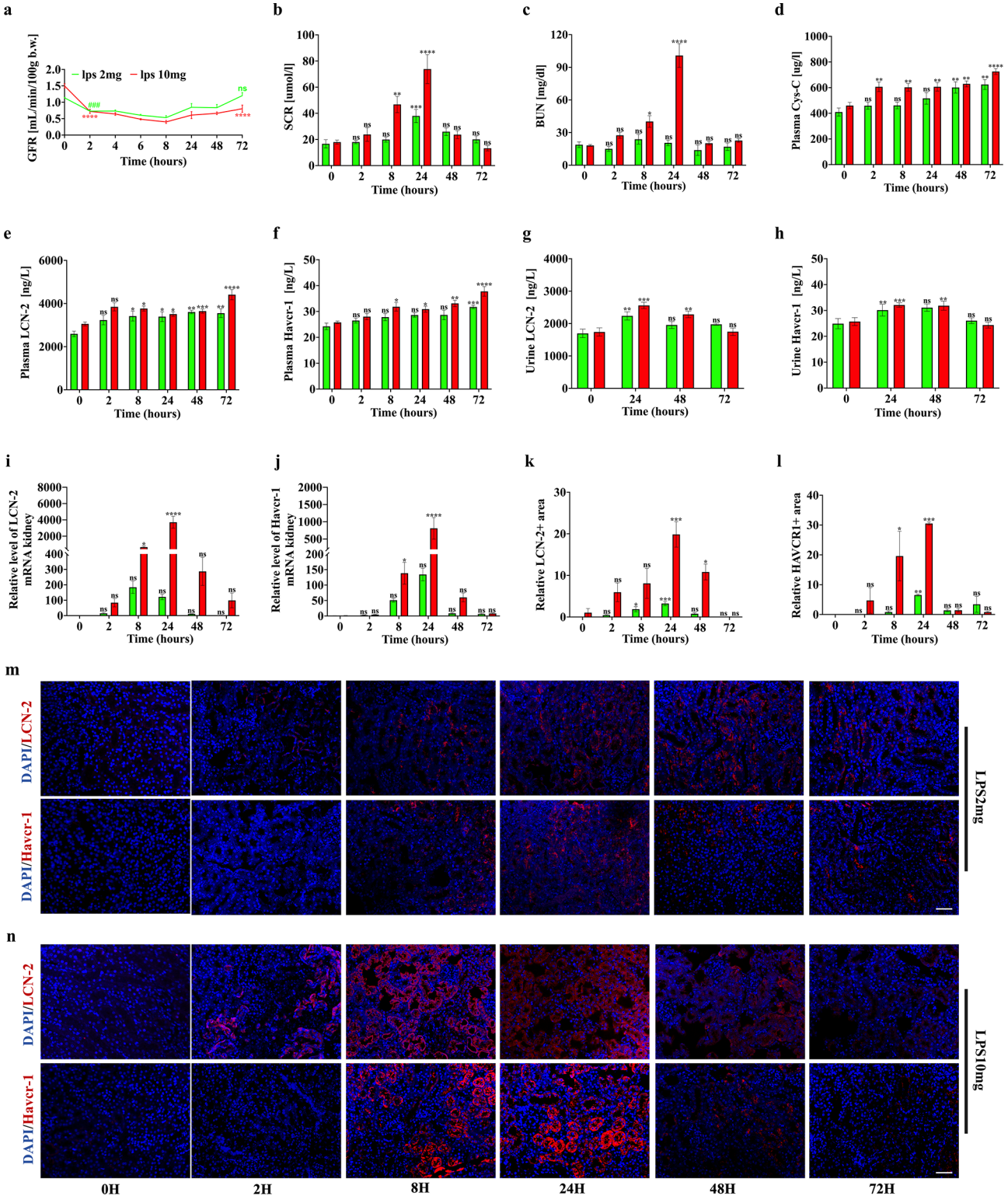


**Fig. 1** Changes in the glomerular filtration rate and other biomarkers in acute kidney injury induced by LPS. (**a**) Dynamic change in glomerular filtration rate at different times after LPS injection ($n=6$). (**b**) Serum creatinine, (**c**) urea nitrogen, (**d**) cystatin-c, (**e**) LCN-2 and (**f**) HAVCR-1 levels at different times after LPS injection ($n=6$). (**g**) Urine LCN-2 and (**h**) HAVCR-1 levels at different times after LPS injection ($n=6$). (**i**) LCN-2 and (**j**) HAVCR-1 mRNA expression levels in mouse kidney at different times after LPS injection ($n=6$). (**k** - **n**) Immunofluorescent staining was used to evaluate the expression of LCN-2 and HAVCR-1 (LCN-2/HAVCR-1; red) in the kidneys. Nuclei were visualized via dapi (blue) staining. The positive areas in the kidneys were analyzed ($n=3$). Bar = 50 mm. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$; Data are presented as mean ± SEM

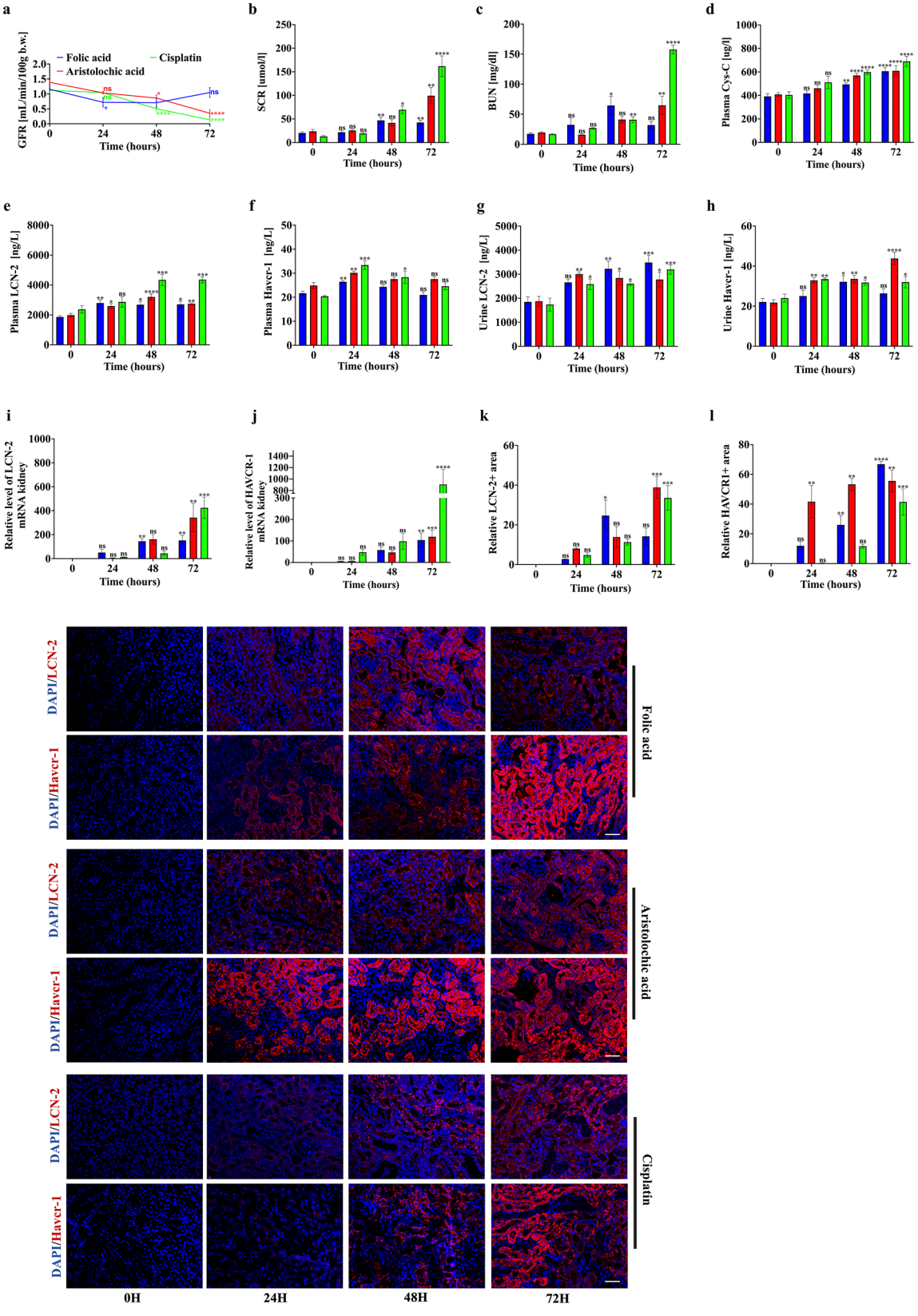


**Fig. 2** (See legend on next page.)

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**Fig. 2** Changes in the glomerular filtration rate and other biomarkers in acute kidney injury induced by folic acid, aristolochic acid and cisplatin. (**a**) Dynamic change in glomerular filtration rate at different times after folic acid, aristolochic acid and cisplatin injection ($n=6$). (**b**) Serum creatinine, (**c**) urea nitrogen, (**d**) cystatin-c, (**e**) LCN-2 and (**f**) HAVCR-1 levels at different times after folic acid, aristolochic acid and cisplatin injection ($n=6$). (**g**) Urine LCN-2 and (**h**) HAVCR-1 levels at different times after folic acid, aristolochic acid and cisplatin injection ($n=6$). (**i**) LCN-2 and (**j**) HAVCR-1 mRNA expression levels in mouse kidney at different times after folic acid, aristolochic acid and cisplatin injection ($n=6$). (**k** - **n**) Immunofluorescent staining was used to evaluate the expression of LCN-2 and HAVCR-1 (LCN-2/HAVCR-1; red) in the kidneys. Nuclei were visualized via dapi (blue) staining. The positive areas in the kidneys were analyzed ($n=3$). Bar = 50 mm. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$; Data are presented as mean ± SEM

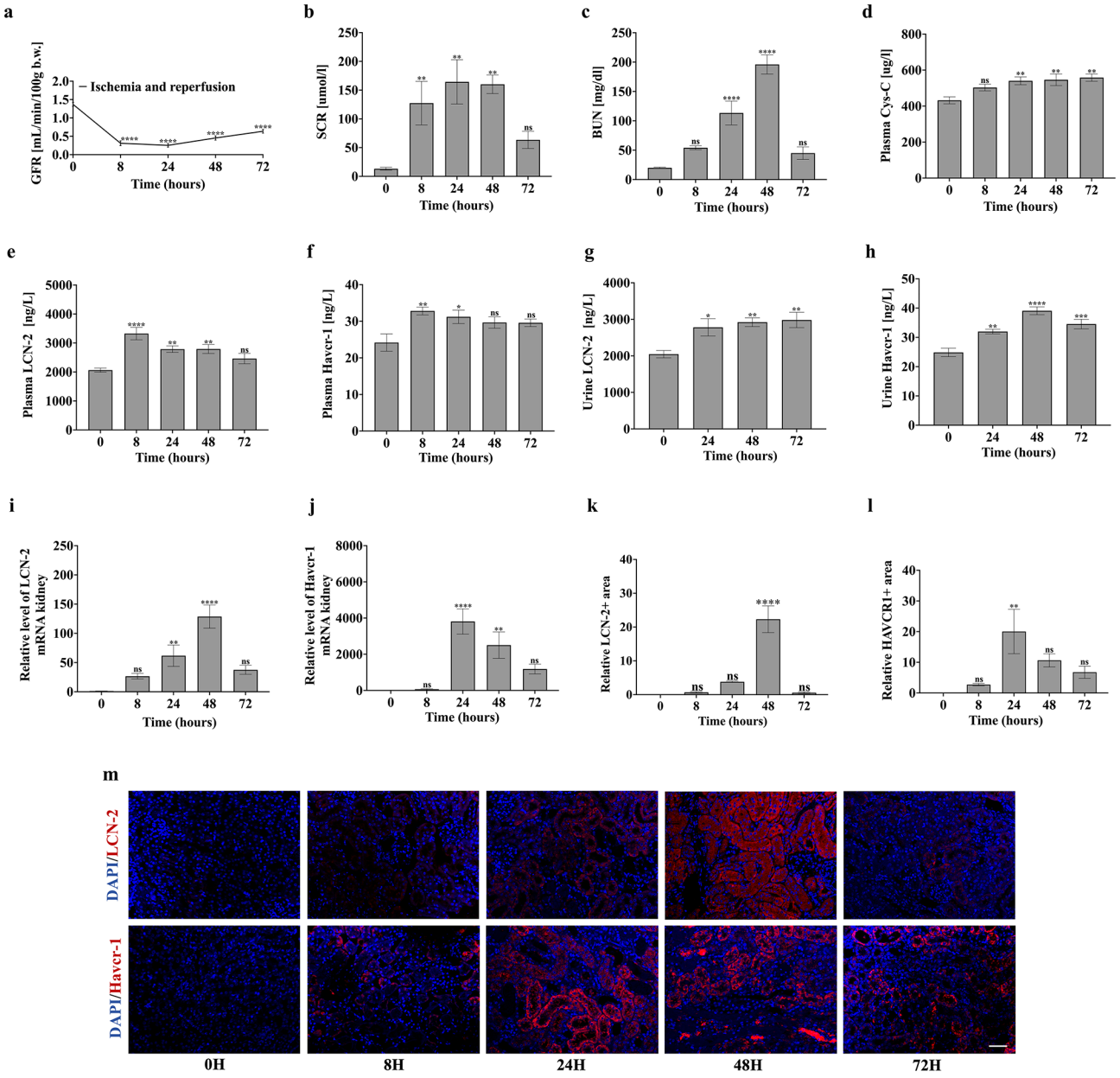


**Fig. 3** Changes in the glomerular filtration rate and other biomarkers in acute kidney injury induced by ischemia–reperfusion. (**a**) Dynamic change in glomerular filtration rate at different times after ischemia–reperfusion ($n=6$). (**b**) Serum creatinine, (**c**) urea nitrogen, (**d**) cystatin-c, (**e**) LCN-2 and (**f**) HAVCR-1 levels at different times after ischemia–reperfusion ($n=6$). (**g**) Urine LCN-2 and (**h**) HAVCR-1 levels at different times after ischemia–reperfusion ($n=6$). (**i**) LCN-2 and (**j**) HAVCR-1 mRNA expression levels in mouse kidney at different times after ischemia–reperfusion ($n=6$). (**k**-**n**) Immunofluorescent staining was used to evaluate the expression of LCN-2 and HAVCR-1 (LCN-2/HAVCR-1; red) in the kidneys. Nuclei were visualized via dapi (blue) staining. The positive areas in the kidneys were analyzed ($n=3$). Bar = 50 mm. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$; Data are presented as mean ± SEM

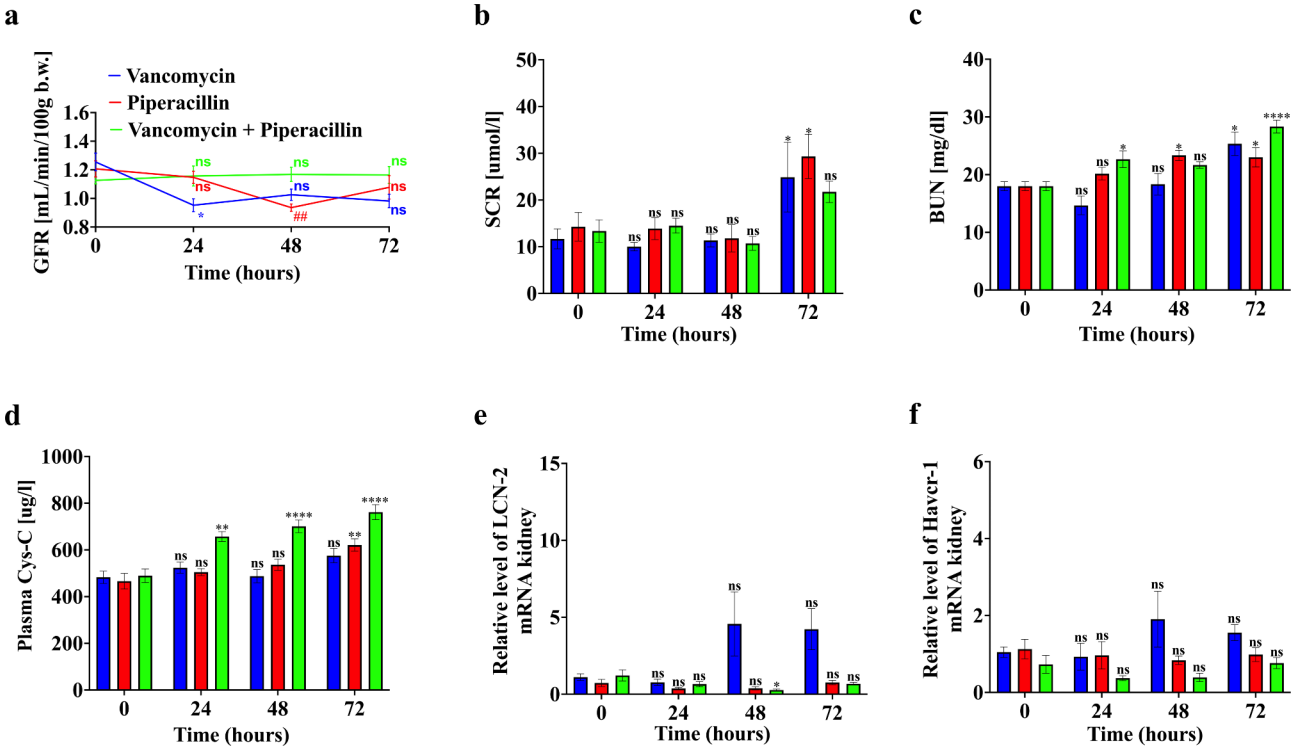


**Fig. 4** Changes in the glomerular filtration rate and other biomarkers in acute kidney injury induced by antibiotics. (**a**) Dynamic change in glomerular filtration rate at different times after antibiotics injection ($n=6$). (**b**) Serum creatinine, (**c**) urea nitrogen, (**d**) cystatin-c levels at different times after antibiotics injection ($n=6$). (**e**) LCN-2 and (**f**) HAVCR-1 mRNA expression levels in mouse kidney at different times after antibiotics injection ($n=6$). *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$; Data are presented as mean ± SEM

## Discussion

The use of daily Scr levels as a surrogate for the GFR not only delays the development of diagnostic methods and successful treatment but also hinders a deeper understanding of the pathophysiology of AKI [7]. Because specific biomarkers of renal injury can be used to monitor specific nephrotoxic. It is necessary to further clarify the specific biomarker elevation and expression patterns related to various nephrotoxins. Percutaneous elimination of FITC-sininistrin can be used on the back of a freely moving rat, and renal function can be reliably measured without the need for blood sampling or laboratory testing. The need for anesthesia and the effect on renal function are eliminated simultaneously [8, 9]. In this study, we measured real-time GFR and compared it with other biomarkers in multiple micde models of kidney injury.

According to our results, all kidney injury biomarkers lagged the changes in real-time GFR during the progression and recovery phases of AKI. With respect to the sepsis-induced acute kidney injury model, we found a significant decrease in 2-hour, which we speculate may be due to a sharp decrease in early renal perfusion; however, the existing information on early renal perfusion in sepsis patients is still controversial [10–12]. Early continuous monitoring could reveal the initial factors involved in the rapid decline in renal function in the early stage of sepsis-induced AKI to guide early fluid resuscitation and the use of vasoactive drugs. As with ischemia–reperfusion AKI, all biomarkers returned to baseline by day 3, except for the GFR. Previous studies have shown that in models of ischemia-reperfusion, the GFR begins to decline as early as 2 h after reperfusion [13], but we did not perform serial measurements over the first 8 h because factors related to anesthesia may affect the measurement of the percutaneous GFR. At present, there is still a lack of clear definition criteria for the recovery of AKI, and the use of these criteria is dependent on the use of the Scr level as a reference [14]. From the results of our preclinical study, this seems to be less reasonable and should be combined with other markers or clinical manifestations to prevent the use of nephrotoxic drugs and prevent the recurrence of AKI or progression to chronic kidney disease (CKD). In the cisplatin-induced AKI, renal function began to decrease on the second day, but the change was very rapid. On the third day, the real-time GFR approached the lowest value, and renal filtration was almost completely lost in some mice.

With respect to the antibiotic-induced kidney injury model, we constructed a model of relatively mild injury. Vancomycin and piperacillin-tazobactam caused varying degrees of decline in renal function in mice, but the

combination of the two drugs did not cause a reduction in the real-time GFR, which seems to be inconsistent with clinical experience [15]. Previously, similar studies showed that vancomycin combined with piperacillin-tazobactam treatment did not lead to significant changes in the real -time GFR in rats, this study suggests that the combination of piperacillin may provide renoprotection [16]. However, in our study, the BUN and Cystatin-c level increased stably, and we were not able to provide a reasonable explanation for the inconsistency between the real-time GFR and biomarker levels. We hypothesized that the gut microbiota might play a role in this process [17]. We will conduct additional studies to explore antibiotic-related kidney damage, including different doses and combinations of antibiotics.

In this study, we applied the technique of percutaneous measurement of the real-time GFR to each mice kidney injury model to determine the changes in renal function over time. However, this study has several limitations. For a sharp decrease in the GFR, we were not able to monitor heart rate, blood pressure or blood flow to the kidneys; therefore, we cannot provide a reasonable explanation. In addition, we found in different batches of the mice, the mice in the same instant strains, weeks, and in the same environment measurement under the condition of the baseline GFR level still exist certain differences, compared with other biomarkers that may be a limitation of the detection method Finally, for the antibiotic model, we did not use any other dose or method of administration.

## Conclusion

In conclusion, the percutaneous real-time glomerular filtration rate technique can be used to dynamically monitor various models of acute kidney injury, and in each model, the glomerular filtration rate changes before other biomarkers. We believe that this technique can be a good tool for predicting the development and recovery of renal function during acute kidney injury.

### Abbreviations

| | |
|---|---|
| AKI | Acute kidney injury |
| GFR | Glomerular filtration rate |
| SCR | Serum creatinine |
| BUN | Blood urea nitrogen |
| NGAL | Neutrophil gelatinase-associated lipocalin |
| KIM-1 | Kidney injury molecule-1 |
| CKD | Chronic kidney disease |

### Acknowledgements

Not applicable.

### Author contributions

Yu Xin wrote the manuscript. Yanqi Liu, Linqiong Liu, Xinran Wang and Dawei Wang conceived and led the study. Yuchen Song, Lifeng Shen, Yuxi Liu, Yuhan Liu Yahui Peng, Xibo Wang and Yang Zhou participated in the animal experiments. Hongxu Li, Yuxin Zhou, Pengfei Huang, Mengyao Yuan and Yu Xiao were responsible for the data analysis. Changsong Wang and Kaijiang Yu revised the text. All the authors read and approved the final manuscript.

### Funding

This study was supported by the National Natural Science Foundation of China-Regional Innovation and Development Joint Fund (U20A20366) and the Natural Science Foundation of Heilongjiang Province (JQ2021H003).

### Data availability

All the data supporting the conclusions presented in this article are included in this published article.

## Declarations

### Ethics approval and consent to participate

Ethical Review of the Use and Welfare of Laboratory Animals at the First Affiliated Hospital of Harbin Medical University No. IACUC: 2023022.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

Received: 7 March 2024 / Accepted: 9 September 2024
Published online: 27 September 2024

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