Urinary L-type fatty acid-binding protein as a new renal biomarker in critical care
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Purpose of review
Acute kidney injury (AKI) remarkably increases the mortality of critically ill patients treated in ICUs. Recently, several renal biomarkers have been developed for the early detection of AKI. We review the potential of urinary L-type fatty acid-binding protein (L-FABP) as a new renal biomarker for AKI diagnosis in critical care.

Recent findings
In the kidney, L-FABP is expressed in renal proximal tubular epithelial cells and shed into urine rapidly in response to renal insults. By using human L-FABP transgenic mice, we reported that urinary L-FABP can detect AKI sensitively and reflect its severity accurately in animal models of AKI and sepsis. In clinical evaluations, the good performance of urinary L-FABP was demonstrated not only in pediatric postcardiopulmonary bypass surgery AKI and contrast media-induced AKI but also in septic shock patients complicated with AKI.

Summary
Recent data suggest that urinary L-FABP can contribute to the development of new AKI diagnostic tools in critical care. Combining with other renal markers such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), optimal threshold determination for distinguishing AKI from chronic renal failure should be explored before translation to the clinical.

Keywords
acute kidney injury, L-type fatty acid-binding protein, urinary biomarker

Introduction
Acute kidney injury (AKI) is a severe complication for critically ill patients in ICUs because AKI significantly increases their mortality [1–4]. However, we do not have any effective drug that has been demonstrated to prevent and treat AKI sufficiently. So far, several clinical investigations failed to develop new AKI therapeutic strategies partly because recruitment of AKI patients in these clinical trials was based on a change of serum creatinine, which cannot reflect the glomerular filtration rate (GFR) of critically ill patients in a nonsteady state [5]. Moreover, serum creatinine cannot detect early renal tubular injury before the decrease of GFR. Therefore, it may be possible that an optimal therapeutic window for new AKI drugs was missed [6]. Earlier detection and more accurate prediction of AKI compared with serum creatinine-based diagnosis are indispensable. Recently, new renal biomarkers including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin 18 (IL-18), and L-type fatty acid-binding protein (L-FABP) have been developed to overcome these problems [7]. In this review, we will focus on urinary L-FABP and discuss its potential as a new renal biomarker in critical care.

Renal expression of L-type fatty acid-binding protein
FABP family is known as intracellular lipid chaperones that transport lipids to the specific component in the cell, although little is known about their exact biological functions and mechanisms of action [8]. There are several different types of FABP and the tissue distribution of FABPs is rather ubiquitous [9] (Table 1). Originally, L-FABP was isolated from rat liver tissue and considered as a free fatty acid carrier protein because it binds selectively to free fatty acid [10,11]. The FABPs have a wide range of different homology from 15 to 70% and L-FABP appears not to have a high homology with other FABPs (25–30%) [12].

L-FABP is expressed not only in the kidney but also in hepatocyte and crypt to villus tip of intestine. In contrast,
2 Renal system

Table 1 Fatty acid-binding protein family

<table>
<thead>
<tr>
<th>Name</th>
<th>Localization</th>
</tr>
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<tbody>
<tr>
<td>L-FABP (liver-type)</td>
<td>Liver, kidney, intestine, pancreas, lung, stomach</td>
</tr>
<tr>
<td>H-FABP (heart-type)</td>
<td>Heart, muscle, kidney, brain, lung, stomach</td>
</tr>
<tr>
<td>I-FABP (intestinal-type)</td>
<td>Intestine, liver</td>
</tr>
<tr>
<td>A-FABP (adipocyte-type)</td>
<td>Adipocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>E-FABP (epidermal-type)</td>
<td>Skin, adipocyte, macrophage, dendritic cell, brain</td>
</tr>
<tr>
<td>II-FABP (ileal-type)</td>
<td>Ileum, ovary, adrenal gland, stomach</td>
</tr>
<tr>
<td>B-FABP (brain-type)</td>
<td>Brain, glia cell, retina</td>
</tr>
<tr>
<td>M-FABP (myelin-type)</td>
<td>Peripheral nervous system, Schwann cell</td>
</tr>
<tr>
<td>T-FABP (testis-type)</td>
<td>Testis, salivary gland, mammary gland</td>
</tr>
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</table>

FABP, fatty acid-binding protein.

the kidney expressed L-FABP and H-FABP. L-FABP was exclusively localized to the cytoplasmic region of proximal tubular epithelial cells, whereas H-FABP was expressed in distal tubular epithelial cells [13,14]. The role of H-FABP in kidney diseases has been reviewed elsewhere [15]. Sweetser et al. [16] reported that rodent kidney does not express L-FABP in normal condition because of the silencing sequence in the upstream of promoter region. We also observed virtually no L-FABP protein expression in the kidney of C57BL/6 mouse in normal and pathophysiological conditions [17]. Because the kidneys of wild-type rodents are equal to deficient condition, we developed human L-FABP transgenic mice with a transgene of human L-FABP containing the whole L-FABP promoter region. The kidney of human L-FABP transgenic mice does not express rodent L-FABP but human L-FABP (‘humanized’ L-FABP transgenic mice) [14,18,19]. L-FABP transgenic mice can be utilized to investigate the role of L-FABP in the kidney by measuring human L-FABP in mouse urine.

Urinary L-type fatty acid-binding protein in animal models of acute kidney injury and sepsis

In the mouse AKI models of cisplatinum injection and renal ischemia–reperfusion, urinary L-FABP of human L-FABP transgenic mice remarkably increased in proportion with the dose of cisplatinum or ischemic time. Severity of histological injuries can successfully be monitored by measuring urinary L-FABP [20,21]. It is of note that increase in urinary L-FABP was observed 1 h after ischemia even in the mild-ischemia (5 min) group. Therefore, urinary L-FABP can detect AKI early and sensitively in these AKI models.

We recently evaluated the utility of urinary L-FABP measurement with sepsis animal models of cecal ligation and puncture (CLP) and intratracheal lipopolysaccharide (LPS) injection [22]. Severity of sepsis was modified by different needle sizes and LPS doses, respectively. In the CLP model, the animals did not become clinically sick at 3 h after the surgery; however, urinary L-FABP levels in the severe (18-G needle) and the less severe (21-G) sepsis groups were significantly higher than in the sham-operated animals. Six hours after surgery, urinary L-FABP levels were significantly different between the severe and the less severe sepsis group (Fig. 1). These data indicate that urinary L-FABP can detect abdominal sepsis early (3 h) and predict severity accurately (6 h). In the intratracheal LPS injection model, urinary L-FABP increased 6 h after injection and returned to the baseline 18 h in the 50 μg LPS group and remained at a higher level in the 200 μg LPS group. Increased urinary L-FABP by intratracheal LPS injection was also able to detect the severity of pulmonary sepsis.

Figure 1 Urinary L-type fatty acid-binding protein in a mouse cecal ligation and puncture model

(a) Survival analysis showed the difference of severity between the 18-G needle puncture group and the 21-G group. (b) Urinary L-FABP level in sepsis induced by CLP. *P < 0.05 versus sham; #P < 0.05 versus the 21-G needle puncture group. L-FABP, L-type fatty acid-binding protein.
started to increase after percutaneous coronary intervention even in the patients who did not show any changes significantly. Whereas serum creatinine started to increase after 24–48 h in the AKI patients. Receiver operating characteristic (ROC) curve analysis for post-CPB AKI diagnosis revealed the area under the ROC curve of urinary L-FABP (4 h after surgery) was 0.810, which is an acceptable level for the single predictive biomarker. Univariate logistic regression analyses showed that both bypass time and urinary L-FABP were significant independent risk indicators for AKI. In another clinical study, urinary L-FABP was evaluated in patients injected with a nonionic, low-osmolar type contrast media.[23]. Thirteen of 66 patients had significantly high urinary L-FABP before injections (>15 μg/g cre) and all these patients showed increased serum creatinine, whereas no patient with low baseline urinary L-FABP showed decline of renal function. It is also reported that urinary L-FABP and NGAL were significantly increased after percutaneous coronary intervention even in the patients who did not show any changes of serum creatinine.[24]. These data suggest that urinary L-FABP is a sensitive marker for renal tubular damage and should be monitored intensively before radiocontrast examination.

### Urinary L-type fatty acid-binding protein in clinical settings

As the first clinical evaluation, urinary L-FABP was examined in pediatric postcardiopulmonary (post-CPB) bypass surgery patients[22]. Urinary L-FABP in the AKI patients measured 4 h after the surgery was significantly higher than that in the non-AKI patients, whereas serum creatinine started to increase after 24–48 h in the AKI patients. Receiver operating characteristic (ROC) curve analysis for post-CPB AKI diagnosis revealed the area under the ROC curve of urinary L-FABP (4 h after surgery) was 0.810, which is an acceptable level for the single predictive biomarker. Univariate logistic regression analyses showed that both bypass time and urinary L-FABP were significant independent risk indicators for AKI. In another clinical study, urinary L-FABP was evaluated in patients injected with a nonionic, low-osmolar type contrast media.[23]. Thirteen of 66 patients had significantly high urinary L-FABP before injections (>15 μg/g cre) and all these patients showed increased serum creatinine, whereas no patient with low baseline urinary L-FABP showed decline of renal function. It is also reported that urinary L-FABP and NGAL were significantly increased after percutaneous coronary intervention even in the patients who did not show any changes of serum creatinine.[24]. These data suggest that urinary L-FABP is a sensitive marker for renal tubular damage and should be monitored intensively before radiocontrast examination.

### Urinary L-type fatty acid-binding protein in ICU patients

So far, urinary L-FABP was evaluated using a cross-sectional approach with severe septic shock patients or with established AKI that occurred during a hospital stay.[25,26]. We previously reported a prospective clinical study of urinary L-FABP measurement with septic shock patients with established AKI[21**]. One hundred forty-five septic shock patients who were diagnosed as AKI at the time of ICU admission (established AKI) were analyzed. Urinary L-FABP levels were significantly higher in nonsurvivors than in survivors and a multiple logistic regression analysis incorporating age, sex, mean blood pressure, and five laboratory measurements (blood endotoxin, CRP, WBC, serum creatinine, and urinary L-FABP) revealed that urinary L-FABP was significantly associated with morality. ROC curve analysis revealed the area under the ROC curve of urinary L-FABP was significantly higher compared with the acute physiology and chronic health evaluation (APACHE) II and sepsis-related organ failure assessment (SOFA) scores. Single measurement of urinary L-FABP may be well received by ICU physicians because these scoring systems are rather time-consuming process.

Polymyxin B-immobilized fiber (PMX-F) treatment has been performed to treat severe sepsis in more than 50,000 patients in Japan since 1994, and a meta-analysis demonstrated its efficacy on septic shock treatment.[27]. A prospective multicenter randomized controlled trial on this endotoxin absorption therapy against abdominal septic shock has recently reported its protective effects.[28]. Forty septic shock patients with established AKI who are treated by PMX-F hemoperfusion were analyzed.[29]. Of 40 septic patients, 28 survived and 12 died. Among the survivors, urinary L-FABP levels were reduced by treatment. However, the nonsurvivors showed higher urinary L-FABP levels with smaller decrease after the treatment compared with the survivors (Table 2). These results suggested that urinary L-FABP levels might be able to reflect the severity of sepsis, and also to monitor the effectiveness of treatment.

It should be noted that the accuracy of the biomarkers was not so good in more heterogeneous cohorts.[30]. This fact underscores the necessity of evaluating the usefulness of new renal biomarkers with more heterogeneous populations. Previous clinical evaluations on urinary L-FABP were performed on relatively homogeneous populations such as pediatric post-CPB patients and adult septic shock patients with established AKI. Critically ill adult patients treated in a mixed ICU (medical–surgical ICU) should have various different backgrounds including comorbidity and frequently have no readily apparent onset of renal injury; some patients were admitted to ICU with established AKI, other patients developed AKI after ICU admission. Further investigation with more heterogeneous populations is necessary to confirm the utility of urinary L-FABP as a renal biomarker in critical care.

### Table 2 Changes of blood endotoxin and urinary L-type fatty acid-binding protein by polymyxin B hemoperfusion

<table>
<thead>
<tr>
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<th>Survivors (n = 28)</th>
<th>Nonsurvivors (n = 12)</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Blood endotoxin (pg/ml)</td>
<td>30.5 ± 9.5</td>
<td>4.6 ± 1.2*</td>
</tr>
<tr>
<td>Urinary L-FABP (μg/g cre)</td>
<td>1420 ± 1080*</td>
<td>240 ± 190*</td>
</tr>
</tbody>
</table>

L-FABP, L-type fatty acid-binding protein.

*P < 0.05 versus before.

*P < 0.05 versus nonsurvivor (before).
Conclusion

Biomarker and diagnostic assay development pathways have four different steps: discovery phase, clinical assay development and validation phase, clinical utility determination phase, and commercial development phase [31]. L-FABP and NGAL have already reached the final step. It was recently suggested that an approach establishing a biomarker panel with several blood and urine potential candidate markers can provide better diagnosis than any single marker [32]. Clinical evaluation studies on renal biomarkers also revealed that combination of biomarkers (biomarker panel) will improve their performance for AKI diagnosis in the widely variable clinical settings [32–34]. For instance, combining early and sensitive AKI markers with late but specific AKI markers will enable to diagnose AKI early and accurately.

Moreover, urinary L-FABP and NGAL can monitor the progression of chronic kidney disease (CKD). These renal biomarkers are increased in CKD patients compared with healthy controls [35,36]. Diabetic nephropathy patients showed a modest but significant increase in urinary L-FABP, which was reduced by lisinopril treatment [37*]. Because urinary L-FABP and NGAL show a wide dynamic range, optimal threshold determination will allow us to distinguish de novo AKI from acute-on-chronic renal failure, which consists of approximately 30–40% of AKI patients.

In conclusion, new renal biomarkers that enable the early detection and accurate prediction will promote development of new AKI diagnosis and treatment in critical care. Several promising AKI biomarkers including urinary L-FABP have been identified recently, but their reliability and generalizability must be confirmed in heterogeneous AKI populations before clinical use. Further clinical evaluations with adult critically ill patients in a mixed ICU are needed.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).


This review article describes the development of urinary L-FABP measurement as a new diagnostic tool for AKI and chronic kidney disease. Details about ‘humanized’ L-FABP transgenic mouse are discussed.


In this study, urinary L-FABP measured at early time-point could predict the final outcome of histological severity and glomerular filtration rate measured by FITC-labeled inulin injection in two mouse AKI models of cisplatin injection and renal ischemia reperfusion.


Urinary L-type fatty acid-binding protein

Doi et al.

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