Background in Context for Cardiorenal Diseases:

Possibilities of the New Biomarker, L-FABP

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A growing recognition of chronic kidney diseases as risk factors

Nagai: As Japan is an aging society, arteriosclerosis and other circulatory system diseases are on the rise, including numerous kidney disorders. For dialysis patients, additional afflictions such as arteriosclerosis or heart conditions can have an impact on prognoses. People with bad kidneys face a worsening of heart conditions, and the same holds true vice-versa: those with bad hearts have worse kidney problems. Heart and kidney diseases have become reciprocal risk factors for complications in each other.

Kimura: That's right. We have known about the heart-kidney relationship for quite some time now. We are now trying to advance this concept by thinking in terms of "CKD"—chronic kidney disease. CKD is diagnosed when any of the following occur independently, or when both have continued for three months or longer: the first is when laboratory findings indicate a kidney disorder—such findings include abnormalities in urine, images (X-rays, etc.), blood, pathology, and so on. The second is when the glomerular filtration rate, or GFR, has been less than 60 mL/minute/1.73 m^2 for three months or longer. With this definition of CKD in hand, we are trying to screen for those at a high risk for problems like coronary heart disease, stroke, peripheral arterial disease (PAD), kidney failure, etc.

In addition, when urinary albumin and urinary protein test positive, and are also increasing, it means the risk for these kinds of diseases is higher. Thus, risk factors for cardiovascular diseases can be easily quantified in a way that's easy to understand for both non-specialist doctors and patients alike. We are conducting a CKD fact-finding survey of 10,000 outpatients in Kanagawa Prefecture who have been making hospital visits for a year or longer, performing urine and serum creatinine tests. Although CKD frequency was thought to be around 13% nationwide, our findings show there are two or three times that many patients. We are even finding high-risk individuals among those patients seeking medical attention for issues other than kidney dysfunction, such as hypertension or lipid metabolism disorders.

Nagai: CKD has been associated with lifestyle-related diseases. It is particularly associated with inadequate self-management of diabetes and high blood pressure. This means that, to some extent, CKD can be controlled and even prevented. Look at chronic glomerulonephritis (CGN), which was once quite common—hasn't that indeed declined?

Kimura: It has declined, yes. Meanwhile, kidney disease patients with a history of diabetes or hypertension have been steadily increasing.

Nagai: Yes, and these kinds of diseases generate substantial medical expenses. Dialysis costs ¥6 million per year, and here, too, complications such as heart disease, etc., can easily occur. We have to put more effort into preventing lifestyle-related diseases. A major goal is to help prevent patients with metabolic syndrome, diabetes, and hypertension from facing more severe diseases, and the key is to prevent heart and kidney diseases. What do you think?

Kimura: CKD is indeed a notion that enables us to discover people in need of speedy medical intervention by checking indicators like urinary proteins and GFR. In addition, CKD as a concept is easy to understand and illustrates that controlling urinary protein decline and GFR decline can lessen the risk of heart- and kidney-related diseases, and that these things are measurable.

Nagai: One fundamental idea in diagnostics is that a decline in kidney function indicates a problem with the heart; but, with the addition of the CKD concept, there is now also an awareness that guidance must be provided to patients at an earlier stage, when their GFR is beginning to decline.

Kimura: That's right. The feeling before now had been that serum creatinine at 1.6 or so meant a light kidney disorder, which was not taken very seriously. Now we know that when calculations are made that take into account factors such as age and gender, the GFR may be worse than it seemed at first.

Nagai: But CKD is most important when one considers larger groups. For individual patients, it is difficult to clarify the issue using just CKD alone, right?

Kimura: Correct. CKD is useful when screening groups, but treatment plans should be created on a case-by-case basis for individual patients.

Nagai: One has to be careful when reading clinical test values for a variety of reasons: for example, serum creatinine does not rise as readily in women, due to their relatively low muscle masses. Special care also has to be taken with the elderly. For GFR, inulin clearance is the most accurate indicator, since even when individual patients share the same kind of serum creatinine levels, one patient's inulin clearance value may be as much as twice that of another's. A patient's serum creatinine may rise for other reasons, too, such as when they don't feel well, or in the summer, when dehydration can occur. Doctors must also take such factors into consideration.

Kimura: Diagnostic guidelines for CKD released in June 2012 included, in addition to serum creatinine, a predictive equation using an indicator called cystatin C, which does not rely on muscle mass. It is recommended that this be measured for persons who have muscle masses outside the standard range. This is also covered by Japanese national health insurance.

Nagai: A combination of various indicators must be used. Physicians should incorporate their own experience and intuition into their diagnoses. As medical science progresses, probability theory—likelihoods and percentages—becomes more and more important. One cannot entirely clarify the causes and conditions of a disease with just a limited number of factors. What's important is the idea of division into sub-groups. While it is essential that a concept like CKD be used as an indicator, individual treatment requires consideration of multiple indicators in combination.

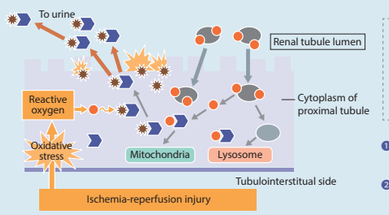
Further clarification of the relationship between free fatty acids and kidney disease progression

Nagai: The kidneys are organs that separate the external environment and the internal environment—items the body doesn't need are excreted in urine. Also, they collectively serve as a "command tower" to maintain homeostasis, identifying various stressors and giving instructions to other bodily organs. We have learned from our research that the kidneys detect heart stress, and that the kidneys issue commands to inhibit that stress. It appears that the kidneys control chronic inflammation in other organs.

Kimura: That's certainly very interesting.

Nagai: It seems that the kidney's epithelial cells work as stress sensors, and that Kruppel-like factor 5 (KLF5) and downstream physiological substances protect the heart. When kidney function is suppressed, the kidneys can no longer protect the heart, and

Fig. 1 L-FABP urinary excretion within proximal tubule cytoplasm



❶Reactive oxygen generated due to peritubular ischemia/reperfusion injury change free fatty acids to fatty acid peroxides (lipoperoxides), which are highly toxic to cells.
❷L-FABP binds with these lipoperoxides, and is excreted outside of cells. Thus, it is thought that L-FABP is "renoprotective"—it works to protect the kidneys.

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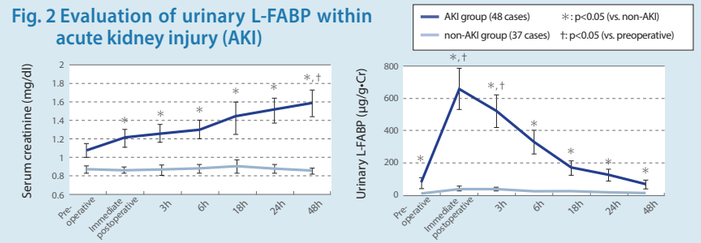
Fig. 2 Evaluation of urinary L-FABP within acute kidney injury (AKI)



(Data submitted by Katsutomi Matsui, Division of Nephrology and Hypertension, Department of Internal Medicine, St. Marianna University School of Medicine)
Source: Matsui K *et al*. Usefulness of urinary biomarkers in early detection of acute kidney injury after cardiac surgery in adults. *Circ J*. 76 213-220 (2012)

inflammation intensifies, increasing the likelihood of heart failure. Organs in the body are interconnected in this way.

Kimura: And it's by the same logic that heart failure is more likely to occur when the renal function of a dialysis patient declines.

Nagai: We think that arteriosclerosis proceeds more readily when the anti-inflammatory functions of the kidneys decline, which makes it difficult to suppress inflammation. However, inflammation does not always lead to a functional disorder. In fact, if some kind of light inflammation does not occur when an organ is under strain, the biological system can sometimes break down. In other words, organs can experience inflammation as a productive response to stress. Yet when that happens repeatedly, structural changes begin to occur, and function gradually declines, which itself can also be a cause of stress.

Kimura: And so aging and habits of daily life can also have an impact, right?

Nagai: Right. As people age, their stress response patterns change. The same goes for kidney function: serum creatinine levels gradually rise. More research is needed to understand how genetic and environmental factors are involved in these changes. As the analysis of the human genome moves forward, rare but dangerous genetic mutations will surely be discovered. Then it may become possible to identify subgroups using CKD that cause heart disease. To understand the significance of such parameters, however, we must keep an eye on long-term prognoses.

Kimura: My own focus is on the inflammation that occurs in renal tubules and their interstitial periphery. The problem was why kidney disorders progress in people who have high urinary protein levels. Kidney disorders lead to leakage of proteins from glomeruli, and when that happens, proximal tubule epithelial cells incorporate those proteins via endocytosis. Accordingly, it was thought that due to some mechanism, inflammatory mediators are released from renal tubule epithelial cells into the interstitial medium, and that inflammation then occurs in the tubulointerstitium. To investigate the mechanism for this, we focused on the free fatty acids attached to the albumin. When bovine albumin is injected into a mouse abdominal cavity, it enters the bloodstream via the peritoneum, following which urinary protein comes out and an interstitial disorder occurs[1]. Yet when albumin in which the free fatty acids have been artificially delipidized is injected into the abdominal cavity, the inflammation is kept at a very light level. So we learned that the fatty acids bound to the albumin are involved in the occurrence and progression of interstitial tubular disorders involving urinary proteins.

Nagai: Fatty acids bind to toll-like receptors, and activate natural immunity. We reported that when we added the saturated fatty acid palmitic acid, inflammation occurred within the islets of Langerhans in the pancreas, and insulin secretion declined; we believe there may be a connection to type 2 diabetes[2]. We also believe that fatty acids can activate natural immunity within other organs as well.

Kimura: Renal tubule interstitial disorder occurs due to the burden from the albumin that is bound with fatty acids. Even with diabetic-related kidney disorders, people with large amounts of urinary protein have large quantities of fatty acids in their urine. Of these fatty acid-bound proteins, we focused this time on the L-type fatty acid binding protein (L-FABP), which is abundantly expressed in proximal tubule epithelial cells. L-FABP promotes fatty acid metabolism, and works to maintain the homeostasis of fatty acids within proximal tubule epithelial cytoplasm (Fig. 1). When there are high levels of urinary protein, L-FABP expression is enhanced within proximal tubules, and urinary excretion is also elevated. Aside from urinary protein issues, there are disease conditions involving a fatty acid burden on proximal tubules—ischemia, renal toxins, and so on—where L-FABP is induced in the proximal tubules, and its excretion in urine is elevated[3]. When one observes the urine of patients with reduced kidney functions pre- and post- cardiac catheterization, there is an increase in L-FABP excretion before the increase in serum creatinine (Fig. 2). In other words, by monitoring the amount of urinary L-FABP, one can make early predictions about the occurrence and worsening of both CKD and acute kidney injury (AKI) alike. When urinary L-FABP is used in combination with urinary albumin, kidney disorder can be predicted with fairly high accuracy. So this is an example of using a combination of indicators as you discussed earlier.

Nagai: We need more research assessing long-term prognoses, especially concerning connections to cardiovascular events.

Kimura: We measured the urinary L-FABP, albumin, and N-acetyl-β-D-glucosaminidase (NAG) of diabetes patients over a five-year period, and compared those whose renal functions worsened with those whose did not. From the results, we learned that L-FABP is an early-stage marker of the risk of CKD progression. Also, in a report from the Steno Diabetes Center in Denmark, in the results of a 15-year tracking study of type 1 diabetes, it was shown that urinary L-FABP at the time of initial testing correlated with the expression of urinary albumin[4]. While conducting joint research with St. Marianna University School of Medicine's Department of Internal Medicine, Division of Cardiology, we noted that people with triple-vessel disease in their coronary arteries had high L-FABP, but there were almost no differences in other indicators such as albumin and NAG.

Nagai: Among our heart catheter patients, too, many who had a high annual percentage decline in GFR also had triple-vessel disease and severe coronary lesions. Can L-FABP be easily measured?

Kimura: Sufficient data has been collected for L-FABP just as for CKD and AKI, so it is now covered by Japanese national health insurance.

Nagai: In the case of diabetes, for example, even when there are stable test values, myocardial infarction can occur. Cardiologists believe that a heart attack may occur at any time in people who have had diabetes for a long time. Minute changes in electrocardiograms and symptoms are monitored closely. Experience is required to acquire the skills to perform that work, and the introduction of new markers would have enormous significance.

Kimura: When clinicians feel there is cause for concern, it would be very useful to have a marker that can verify those feelings. It will become necessary to treat patients while monitoring such markers. Risk groups can then be stratified, and personalized treatments can be prescribed accordingly.

Nagai: It would be great if L-FABP could serve to reinforce the "art" of medicine, which is something not reflected in conventional laboratory test values. ■

Reference

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