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Original article

A newly developed kit for the measurement of urinary liver-type fatty acid-binding protein as a biomarker for acute kidney injury in patients with critical care

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ABSTRACT

In recent years, it has been reported that the urinary level of Liver-type fatty acid-binding protein (L-FABP) serves as a useful biomarker for diagnosing acute kidney injury (AKI) or sepsis complicated by AKI. However, because the urinary level of L-FABP is currently measured by enzyme-linked immunosorbent assay (ELISA), several days may elapse before the results of the measurement become available. We have newly developed a simplified kit, the Dip-test, for measuring the urinary level of L-FABP.

The Dip-test was measured at 80 measurement points (22 points in noninfectious disease, 13 points in SIRS, 20 points in infectious disease, and 25 points in sepsis) in 20 patients. The urinary L-FABP levels as determined by ELISA in relation to the results of the Dip-test were as follows: 10.10 ± 12.85 ng/ml in patients with a negative Dip-test ([-] group), 41.93 ± 50.51 ng/ml in patients with a ± test ([±] group), 70.36 ± 73.70 ng/ml in patients with a positive test ([+] group), 1048.96 ± 2117.68 ng/ml in patients with a 2 + test ([2+] group), and 23,571.55 ± 21,737.45 ng/ml in patients with a 3 + test ([3+] group). The following tendency was noted: the stronger the positive Dip-test reaction, the higher the urinary L-FABP level. Multigroup comparison revealed a significant differences in the urinary L-FABP levels between the Dip-test (−) group and each of the other groups.

In this study, the usefulness of the Dip-test, our newly developed simplified kit for measuring the urinary L-FABP level, is suggested.

1. Introduction

Systemic inflammatory response syndrome (SIRS) [1] is a group of disorders characterized by non-specifically activated immune responses and loss of regulation of cytokine production in response to serious invasion of the living body, which leads to serious multiple organ dysfunction syndrome (MODS) [1]. Because MODS often has a fatal outcome, it is important to understand the patient's general status at the stage of SIRS and undertake suitable intensive treatment, so as to prevent progression to MODS. Patients under emergency and intensive care are likely to develop sepsis along with aggravation of the general condition. In addition, worsening sepsis may be complicated by MODS, including acute kidney injury (AKI), resulting in further aggravation of the general condition.

Fatty acid-binding protein exists in several different isoforms in the heart, brain, small bowel, and liver [2]. Liver-type fatty acid-binding protein (L-FABP) is about 14 kD in molecular weight, and is expressed in the proximal tubular epithelium in humans [3]. In the physiological reaction of the kidney, most of the albumin filtered through the glomeruli is reabsorbed in the proximal tubules with free fatty acids. The reabsorbed albumin is taken up by the lysosomes, while the fatty acids released into the cytoplasm bind to L-FABP and are taken up by peroxisomes to reduce the size. Although lipid peroxides accumulate in the proximal tubules and induce tissue injury in the ischemic state, L-FABP reportedly binds to these harmful lipid peroxides, and is then excreted in the urine [4]. Doi et al. suggested that the urinary L-FABP level might be a

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predictive marker of the severity and mortality of sepsis, and reported that it could serve as a useful biomarker in patients with sepsis complicated by AKI [2]. It has also been reported that urinary L-FABP levels in septic shock patients with AKI were higher than those in healthy persons, in patients with AKI without sepsis, and in sepsis patients without septic shock [5].

At present, urinary L-FABP is measured by enzyme-linked immunosorbent assay (ELISA). ELISA has drawbacks in that one-by-one measurement of samples is difficult, it is a cumbersome technique, and several days may elapse before the results can be obtained. We recently developed a new procedure, Point of Care Testing (POCT), using the simplified kit, Dip-test (CMIC Co Ltd, Tokyo, Japan) for determination of the urinary L-FABP. Rapid qualitative measurement of urinary L-FABP is possible by this method, and to evaluate its usefulness, the results obtained using this test were compared with quantitative measurement of the urinary L-FABP by ELISA.

2. Materials and methods

2.1. Patients

Consent from the patients or their families and approval from the ethics committee of the Iwate Medical University were obtained prior to the initiation of this study. This study was a prospective observational study involving 20 patients (12 men and 8 women, with a mean age of 64.1 ± 20.4 years) who were admitted to the Critical Care and Emergency Center, Iwate Medical University between April and December 2013. Minors (less than 20 years of age) and patients who had received blood purification in critical care (e.g., endotoxin adsorption therapy) at another institution prior to admission at our institution were excluded from this study.

SIRS and sepsis were diagnosed according to the criteria of the Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee [1].

The indices to assess the severity of the critical condition of the patients were the Acute Physiology and Chronic Health Evaluation (APACEH II) score [6] and the Sequential Organ Failure Assessment (SOFA) score [7]. These scores were determined jointly by the same two or more doctors who were qualified as both infection control doctors and as emergency and critical care specialists and clinical research coordinators.

2.2. Measurement methods

2.2.1. Structure and principle of measurement by Dip-test

The principle of measurement by this method is immunochromatography. In this method, a sample dropped on the sample pad is transferred by the capillary phenomenon through the conjugation pad, and the target antigen binds to the membrane through antigen–antibody reaction during the process of its permeation in the antibody solid-phase membrane. The binding is confirmed by visual observation. More specifically, the procedure is as follows. First, a urine sample containing L-FABP (the target antigen) is dropped on to the sample pad. By the capillary phenomenon, the urine sample is transferred to the conjugation pad, and the target antigen binds to the monoclonal antibody directed against human L-FABP (A) + gold colloid particles (labeled antibody. Gold particles: for development of red color) preliminarily solid-phased on the conjugation pad, to form an antigen–antibody complex. Then, the complex is transferred to a subjacent membrane by the capillary phenomenon, where it binds to the primary antibody (B) (antigen-specific antibody) at a different site in A that was preliminarily solid-phased in a position to produce a red line (sandwich method). Thus, a red line (sample detection site: test line) is obtained. On the other hand, for the control line (control detection site), the preliminarily solid-phased secondary antibody (C) binds to A + gold colloid particles to develop a red color, and presents a red line (control line).

The pretreatment liquid is used to enhance immune recognition. L-FABP excreted in the urine does not exist in the free form, but is bound to protein. For its detection, the bound protein is degenerated by the pretreatment liquid and removed.

2.2.2. Biomarker measurement

The day on which the patient was admitted to the hospital was defined as day 1, and urine samples were collected on days 1, 2, 3 and 5. A 100-μL aliquot of the urine sample was infused in a microtube containing the pretreatment liquid, and mixed throughly by gentle inversion. The sample was then suctioned to reach the red line shown on the attached dropper, 3 drops of the sample were instilled in the Dip-test kit, and the urinary L-FABP level was measured by visual observation in 15 min (Figs. 1 and 2). The remaining urine sample was cryopreserved at −80 degrees C. Urinary L-FABP levels were quantitatively determined by ELISA (CMIC Co Ltd, Tokyo, Japan) [8].

2.2.3. Statistical analysis

The results obtained were expressed as mean ± standard deviation (SD). Normality was evaluated by the Shapiro–Wilk test. When comparing groups, the Mann–Whitney *U*-test was used for non-parametric data. For multigroup comparison, the Mann–Whitney *U*-test with Bonferroni's correction and the Steel–Dwass test were used. Differences were regarded as statistically significant at $p < 0.05$. The statistical software JMP11 (SAS Institute Inc., Cary, NC, USA) and SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) were used for these statistical analyses.

3. Results

For the 20 emergency patients admitted to our institution, the urine samples collected on days 1, 2, 3 and 5 were subjected to measurement at a total of 80 measurement points. Table 1 shows the clinical characteristics of the patients. The Dip-test was measured at 80 measurement points (22 measurement points in noninfectious disease, 13 measurement points in SIRS, 20 measurement points in infectious disease excluding sepsis, and 25 measurement points in sepsis including severe sepsis and septic shock) in 20 patients (Table 2). The urinary L-FABP level was determined by ELISA at each measurement point. The urinary L-

Fig. 1. A microtube containing blue pretreatment liquid (left), a dropper that has a red line on it (middle), and the main unit of the kit (right).