Development of a point-of-care assay system for measurement of presepsin (sCD14-ST)

Yoshikazu Okamura *, Hiroyuki Yokoi

Research and Development Division, Mitsubishi Chemical Medience Corporation, Japan

A R T I C L E   I N F O

Article history:
Received 28 April 2011
Received in revised form 25 July 2011
Accepted 26 July 2011
Available online 3 August 2011

Keywords:
Presepsin
sCD14-ST
CLEIA
Sepsis
PATHFAST
PCT

A B S T R A C T

Background: The soluble CD14 subtype (sCD14-ST: renamed as presepsin) is a novel soluble CD14 molecule that is useful for diagnosing sepsis because sCD14-ST levels increase specifically in sepsis patients.

Methods: A fully automated PATHFAST® Presepsin assay system based on a chemiluminescent enzyme immunoassay was developed for detecting presepsin in human whole blood.

Results: The limit of blank, limit of detection, and limit of quantification were 2.33, 13.4, and 47.6 pg/ml, respectively. The assay linearity was achieved up to 20,000 pg/ml. Intra-assay imprecision was 3.4–4.8% for plasma and 2.7–7.1% for whole blood. Within-run imprecision and total imprecision for plasma were 3.6–4.4% and 5.2–6.5%, respectively. No interference was observed with bilirubin, hemoglobin, lipids, triglyceride, or rheumatoid factors. The reference intervals (95% percentile, n=127) were 333 pg/ml for plasma and 314 pg/ml for whole blood. The PATHFAST® Presepsin assay correlated well with a previously reported two-step presepsin ELISA (r=0.984, n=40). Furthermore, the concentration of presepsin was significantly higher in the sepsis group than in the healthy group.

Conclusion: The PATHFAST® Presepsin assay performed well and can be used for point-of-care.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Since the definition of systemic inflammatory response syndrome (SIRS) was proposed in 1991, many clinical trials on sepsis diagnosis and treatment have been conducted using the definition of sepsis given by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [1]. Many studies have reported that early treatment of sepsis using appropriate antibiotics improved the prognosis and increased the survival rate in severe sepsis or septic shock patients [2–4]. Blood culture is frequently used as the “gold standard” diagnostic method for sepsis. However, it usually takes 3 to 7 days to obtain the results and frequently yields low positive results [5]. Therefore, the general practical medical treatment used for sepsis is based on the doctor’s own experience (empiric therapy). From this point of view, a method for diagnosing sepsis with a short turnaround time is crucial.

Various biomarkers have been studied for diagnosing sepsis [6]. Currently, procalcitonin (PCT) is used as a marker to diagnose sepsis or severe sepsis. In comparison to other markers that have traditionally been reported, PCT gives a high rate of specificity for sepsis diagnosis [7]. However, the concentration of PCT in the human blood is elevated in various conditions, such as in severe trauma, surgical invasive procedures, and critical burn injury, which leads to SIRS. It is also necessary to be aware of false-positive results [8]. Therefore, more reliable biomarkers for the diagnosis of sepsis are needed.

Presepsin, which is approximately 13 kDa, has been identified as a protein whose levels increase specifically in the blood of sepsis patients. Presepsin is a more specific and sensitive marker for the diagnosis of sepsis compared with interleukin-6 (IL-6) and PCT [9]. Presepsin concentrations in blood were increased faster than PCT and CRP in sepsis patients [9,10]. Additionally, the measurement of presepsin concentrations is useful for evaluating the severity of sepsis and also for monitoring the clinical responses to therapeutic interventions [11,12]. The previously reported sandwich enzyme-linked immunosorbent assay (ELISA) was not convenient and took a long time to obtain results [9,13].

We have developed a new, highly-sensitive, fully automated PATHFAST Presepsin assay system based on the chemiluminescent enzyme immunoassay (CLEIA) principle, which can be used to analyze whole blood samples. In this study, we evaluated the analytical and clinical performance of this assay and its usefulness in the early diagnosis of sepsis.

2. Material and methods

2.1. Human serum and plasma

The serum, heparinized plasma, heparinized whole blood, and EDTA plasma samples used in the analytical evaluation were from Golden West Biological, Inc. (Temecula CA) and PromedDx (Norton,
MA), or obtained from volunteers within our laboratory. The serum and heparinized plasma samples used in the clinical evaluation were from Discovery Life Sciences (Los Osos CA) and ProMedDx. All the samples were obtained under informed consent rules applicable at each of the facilities.

2.2. PATHFAST Presepsin

The PATHFAST analyzer has previously been described [14]. The PATHFAST Presepsin assay contains magnetic particles coated with mouse monoclonal antibodies and alkaline phosphatase (ALP)-labeled rabbit polyclonal antibodies. Mouse monoclonal antibodies, rabbit polyclonal antibodies, and the recombinant human presepsin antigen were obtained from Mochida Pharmaceutical Co. Ltd. (Tokyo, Japan) [15,16].

The magnetic particles (JSR, Tokyo, Japan) were coated with mouse monoclonal antibodies. The ALP-labeled rabbit polyclonal antibodies were obtained by conjugating Fab’ fragments of the antibodies with ALP using the maleimide-hinge method. The concentration of the presepsin calibrators was assigned by amino-acid composition analysis. CDP-Star was used as the chemiluminescent substrate and was purchased from Applied Biosystems (MA, USA).

Presepsin in the specimen binds to the anti-presepsin antibodies to form an immunocomplex with the ALP-labeled antibodies and the antibody-coated magnetic particles. After removal of the unbound ALP-labeled antibodies, a chemiluminescent substrate was added to the immunocomplex. After a short incubation period, the luminescence generated by the enzyme reaction was detected in order to calculate the concentration of presepsin in the samples. The assay time was 17 min using a sample volume of 100 μl. The entire procedure was automatically performed on the PATHFAST analyzer.

2.3. Assay

The two-step presepsin ELISA was obtained from Mochida Pharmaceutical Co. Ltd. (Tokyo, Japan) [9]. The PCT concentrations were measured using Elecsys® B·R·A·H·M·S PCT, which was from Roche (Tokyo, Japan).

2.4. Statistical analysis

The statistical analysis was performed using Analyse-it® (ver. 2.11) software (Analyse-it software Ltd, UK). Regressions were determined using the Passing Bablok regression procedure [17]. Significant differences were determined using the Mann–Whitney test. A P<0.05 were considered significant.

3. Results

3.1. Sensitivity

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) values were calculated according to CLSI EP17-A [18]. The LoB was estimated with the results from n=60 measurements of a zero standard. The LoB was 1.95 pg/ml (non-parametric) and 2.33 pg/ml (parametric). The LoD and LoQ values were studied using five level samples (21.7, 32.7, 47.6, 75.7, and 127 pg/ml) that were prepared from heparinized plasma samples with recombinant human presepsin antigen. Each samples were assayed triplicately per day for 7 days (n=21/each level). The LoD was 13.4 pg/ml (parametric), and the LoQ was 47.6 pg/ml (10% CV).

3.2. Linearity

A heparinized plasma sample supplemented with 20,902 pg/ml recombinant human presepsin antigen was diluted proportionally in 4 steps of 1+9, 2+8... 8+2, 9+1 using presepsin-free plasma. The mean of the measured concentration at each level was compared to the expected concentration. The linearity of this assay was 94.7–104.6% (Fig. 1).

3.3. Intra-assay imprecision

Four heparinized plasma samples containing recombinant human presepsin antigen were assayed in 20 replicates within the same day to obtain intra-assay imprecision. The CVs for the intra-assays were 3.4–4.8% for the heparinized plasma sample (183–8766 pg/ml) and 2.7–7.1% for the heparinized whole blood sample (113–9186 pg/ml) (Table 1).

3.4. Total imprecision

The heparinized plasma samples containing recombinant human presepsin antigen were kept frozen until analysis. Samples were assayed in duplicate over 20 days, with 2 runs per day (n=80/level) according to CLSI EP5-A2 [19]. Within-run imprecision and total imprecision for the plasma (389–13,006 pg/ml) were 3.6–4.4% and 5.2–6.5%, respectively. (Table 2).

3.5. Interference with endogenous substances

Heparinized plasma samples (about 400 pg/ml) containing free-form bilirubin (8 to 40 mg/dl), conjugated-form bilirubin (8 to 40 mg/dl), hemoglobin (120 to 600 mg/dl), lipids (400 to 2000 FTU), triglyceride (200 to 1000 mg/dl), and RF (100 to 500 IU/ml) were prepared. The free-form and conjugated-form bilirubin, hemoglobin, lipids, and RF were prepared from interference Check A Plus and Interference Check RF (Sysmex, Kobe, Japan). The triglyceride was prepared from the intralipid®
20% (TERUMO, Tokyo, Japan). The recoveries of presepsin in the interference study with the free-form bilirubin, conjugated-form bilirubin, hemoglobin, lipids, triglyceride, or RF were 93.3–105.4%, 97.0–105.6%, 94.5–103.3%, 92.4–104.7%, 91.1–105.5%, and 97.4–102.9%, respectively.

3.6. The matrix effect for sample types

The correlations between sample types were evaluated. Heparinized plasma, EDTA plasma, and serum were collected from each individual. The samples were prepared by adding recombinant human presepsin antigen to each of the specimens. The correlation between the heparinized plasma and the EDTA plasma was $Y = 1.00X + 1.53$ and $r = 0.999$, $n = 23$ (Fig. 2, A). The correlation between the heparinized plasma and the serum was $Y = 0.99X + 38.8$ and $r = 0.998$, $n = 30$ (Fig. 2, B). The matrix effect was not observed for each sample.

With regard to the whole blood samples, the correlation between heparinized plasma and heparinized whole blood was evaluated. Samples were collected from each individual, and antigen-supplemented samples were prepared by adding recombinant human presepsin antigen to them. The correlation between the heparinized plasma and heparinized whole blood samples was $Y = 0.94X + 166$ and $r = 0.992$, $n = 44$ (Fig. 2C).

3.7. The reference interval of the PATHFAST Presepsin assay

Heparinized plasma and heparinized whole blood samples were collected from 127 healthy volunteers and assayed with the PATHFAST Presepsin assay. For heparinized plasma, the presepsin concentrations ranged from 92.7 to 398 pg/ml with an arithmetic mean of 189 pg/ml, and 5th and 95th percentile were 105 and 333 pg/ml. For heparinized whole blood, the presepsin concentrations ranged from 96.4 to 347 pg/ml with an arithmetic mean of 182 pg/ml, and 5th and 95th percentile were 98.3 and 314 pg/ml.

3.8. Comparison between the two-step presepsin ELISA and the PATHFAST Presepsin assay

The correlation between the two-step presepsin ELISA and the PATHFAST Presepsin assay using 40 individually heparinized plasma samples was evaluated. The correlation was $Y = 0.00727X - 26.9$ and $r = 0.984$, $n = 40$. There was a highly significant correlation between the two assays (Fig. 3).

3.9. Presepsin concentrations in the healthy group and the sepsis group

The serum presepsin and PCT levels were compared between the healthy ($n = 20$) and sepsis groups ($n = 20$). The sepsis group was diagnosed with suspected or confirmed sepsis by blood culture. The level of presepsin was $123 \pm 67.5$ pg/ml in the healthy group and $2363 \pm 2161$ pg/ml in the sepsis group; thus, the sepsis group had a significantly higher level of presepsin than the healthy group ($p < 0.0001$) (Fig. 4A). Also, the PCT level was $0.024 \pm 0.019$ ng/ml in the healthy group and $10.98 \pm 28.93$ ng/ml in the sepsis group; thus, the sepsis group had a significantly higher level of PCT than the healthy group ($p < 0.0001$) (Fig. 4B).

Table 2
Total imprecision.

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (pg/ml)</th>
<th>Within-run SD</th>
<th>CV (%)</th>
<th>Total SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>389</td>
<td>17.2</td>
<td>4.4</td>
<td>25.1</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>1026</td>
<td>38.8</td>
<td>3.8</td>
<td>54.7</td>
<td>5.3</td>
</tr>
<tr>
<td>3</td>
<td>5464</td>
<td>195</td>
<td>3.6</td>
<td>282</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>13,006</td>
<td>558</td>
<td>4.3</td>
<td>716</td>
<td>5.5</td>
</tr>
</tbody>
</table>

![Fig. 2. Correlation between the sample types. A: heparinized plasma and EDTA plasma ($Y = 1.00X + 1.53$, $r = 0.999$, $n = 23$), B: heparinized plasma and serum ($Y = 0.99X + 38.8$, $r = 0.998$, $n = 30$), C: heparinized plasma and heparinized whole blood ($Y = 0.94X + 166$, $r = 0.992$, $n = 44$).](image)
4. Discussion

The PATHFAST Presepsin assay system demonstrated a sufficient analytical performance. The sensitivity of PATHFAST Presepsin assay was sufficient to detect the presepsin concentrations of the healthy group. It was higher than the recently reported one-step ELISA [13]. Also, the reference interval of plasma was similar to whole blood on the PATHFAST. The PATHFAST Presepsin assay correlated well with a previously reported two-step presepsin ELISA [9]. However, the regression slope of the correlation between two-step ELISA and PATHFAST Presepsin was 0.00727 due to the difference in calibrator substance and the assigning method of calibrator [13]. The presepsin concentrations measured in the two-step ELISA were estimated based on the use of a large recombinant CD14 antigen (approximately 40 kDa) as standard [15]. The calibrator substance for the PATHFAST Presepsin assay was changed from the recombinant CD14 to recombinant presepsin (13 kDa).

In the clinical performance of the PATHFAST Presepsin assay, the presepsin concentrations was significantly higher in the sepsis group than in the healthy group, as was also observed with PCT. When the cutoff value of presepsin was set at 415 pg/ml, clinical sensitivity was 90.0% and clinical specificity was 100% [20]. When the cutoff value of PCT was set at 0.5 ng/ml, clinical sensitivity was 65.0% and clinical specificity was 100%. These data demonstrated that the PATHFAST Presepsin assay was valuable for the diagnosis of sepsis. According to other clinical studies of PATHFAST, the presepsin concentrations were usefulness for the diagnosis of sepsis/infection in comparison with PCT [20]. However, more study is necessary to establish the cutoff value of presepsin.

The PATHFAST Presepsin assay reveals its result within 17 min. This is earlier than the previously reported presepsin ELISAs [9,13]. Furthermore, the PATHFAST Presepsin assay can be performed using whole blood. Whole blood samples are suitable for use in the emergency room, ICU, and the surgical operation room. Thus, the turnaround time for generating results using the PATHFAST Presepsin assay is within 1 h. The 2008 Guidelines of the Surviving Sepsis Campaign (SSC) recommended that a specific anatomic site of infection should be established as rapidly as possible within the first 6 h of presentation and that antibiotic treatment must be started within 1 h after the recognition of severe sepsis [21]. After the onset of sepsis, the timing of the diagnosis and treatment is crucial. We demonstrated that the PATHFAST Presepsin assay is a new tool for the early diagnosis of sepsis and can be adapted for this recommendation.

**Abbreviations**

ALP  alkaline phosphatase
CDP-Star disodium 2-chloro-5-(4-methoxySpiro (1,2-dioxetane-3,2′-(S′-chloro)tricyclo[3.3.1.13,7]decan)-4-yl) phenyl phosphate
CLEIA  chemiluminescent enzyme immunoassay
CV coefficient of variation
ELISA enzyme-linked immunosorbent assay
FTU formazin turbidity unit
PCT procalcitonin
POCT point-of-care testing
RF rheumatoid factor
sCD14-ST soluble CD14 subtype
SSC Surviving Sepsis Campaign

References