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LETTER TO THE EDITOR

Diagnostic usefulness of plasma presepsin (soluble CD14-subtype) for diagnosing hemophagocytic syndrome in hematological malignancies

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Secondary hemophagocytic syndrome (HPS) develops predominantly in adults and is triggered by a number of factors; it is a persistent hyper-inflammatory condition associated with activated macrophages and cytotoxic lymphocytes, caused by the failure of homeostatic antigen elimination [1]. Clinical manifestations of HPS include high fever, cytopenias and splenomegaly, and it can lead to multiple organ failure and a high rate of early mortality. Although early diagnosis of HPS is a key to improving prognosis, the current hemophagocytic lymphohistiocytosis (HLH)-2004 criteria, which require that at least 5 out of 8 non-specific findings are met for HPS diagnosis [2], have several disadvantages as follows: some diagnostic findings are often not observed at the onset, some data as soluble interleukin-2 receptor (sIL-2R) may not be available quickly and timely, and some data as cytopenias may be difficult to assess under the conditions of intensive chemotherapy. To overcome these shortcomings, a recent retrospective epidemiologic study reported an HPS scoring system that uses clinical characteristic variables to generate a score representing the probability of having HPS [3]. In contrast, several blood biomarker studies reported serum ferritin levels of >10,000 μg/L [4], the serum sIL-2R to ferritin ratio [5,6], and the serum levels of interferon (IFN)-γ and interleukin (IL)-10 [7], as useful diagnostic biomarkers for HPS. However, the relevance of these biomarkers for the standard diagnostic method for HPS has yet to be determined.

Presepsin (soluble CD14-subtype) has been increasingly reported to be a useful biomarker for sepsis [8]. Recently, Arai et al. showed that human monocytes secrete presepsin in response to a sterile phagocytic stimulus, as well as bacterial phagocytosis, and that serum levels of presepsin in patients with HPS were significantly higher than those in subjects without HPS [9]. Nanno et al. found that a composite model of plasma presepsin and serum sIL-2R levels might serve as a useful prognostic marker for HPS [10]. Based on these observations, we hypothesized that presepsin could be a superior diagnostic biomarker, and undertook a cross-sectional study in which we compared the diagnostic performance of plasma presepsin with that of serum ferritin, sIL-2R, IFN-γ, IL-10, and IL-6.

Between April 2006 and August 2014, we consecutively examined patients whose blood samples at onset of HPS were available in our institution. We diagnosed HPS using the modified HLH-2004 criteria, as previously reported [10,11]. Control blood samples were from patients who had serum ferritin levels of ≥500 μg/L at the onset of febrile neutropenia (FN), and were obtained from subjects who had been enrolled in two previous clinical studies [12,13]. Plasma levels of presepsin were determined using the PATHFAST® Presepsin kit (LSI Medience Corporation, Tokyo, Japan). Serum levels of ferritin and sIL-2R were measured using the Chemilumi ACS-Ferritin II kit and the Siemens Immulyze IL-2R II kit, respectively. Serum levels of all

cytokines were assessed using the Bio-Plex Pro Cytokine Assay® system.

A result was considered to be positive if it exceeded the following values:

314 pg/mL for presepsin, 261 μg/L for ferritin, 466 U/mL for sIL-2R, 124 pg/mL for IFN-γ, 2.0 pg/mL for IL-10, and 9.0 pg/mL for IL-6, according to the respective manufacturer's instructions. The diagnostic performance of each HPS biomarker was compared using receiver operating characteristic (ROC) curves. The best cut-off points were calculated by the Youden index. A statistical significance level of 0.05 was set, and all statistical analyses were performed using IBM SPSS Statistics, version 22.0. We obtained approval for this study from the Human Subjects Review Committee at Osaka City University.

A total of 12 samples from patients with HPS and 31 control samples from 22 patients with FN were eligible and evaluable. The background details of these subjects are shown in Table 1. All patients with HPS were treated with steroids, etoposide, and/or calcineurin inhibitors. Of the 12 patients, at 8 weeks after treatment, 7 achieved a partial response and 5 progressed/relapsed. At last follow-up, only 4 HPS patients had survived, whereas all FN patients completely recovered and survived.

To assess the relevance of each biomarker, Spearman's correlation coefficients between presepsin and ferritin, sIL-2R, IFN-γ, IL-10, and IL-6 were calculated for all samples (n = 43). The correlation coefficients were 0.656 ($P < 0.0001$), 0.718 ($P < 0.0001$), 0.214 ($P =$

0.168), 0.370 (P = 0.015) and 0.467 (P = 0.002), respectively. Levels of presepsin, ferritin, sIL-2R and IL-10, but not IFN-γ and IL-6, were significantly higher in patients with HPS than those in control samples (Table 1).

All ROC curves are shown in Figure 1. The areas under the ROC curves (AUC) for HPS were 0.934 (95% confidence interval [CI], 0.819–1.000; $P < 0.0001$) for presepsin, 0.981 (0.949–1.000, $P < 0.0001$) for ferritin, 0.954 (0.893–1.000, $P < 0.0001$) for sIL-2R, 0.567 (0.371–0.764, P = 0.498) for IFN-γ, 0.777 (0.636–0.918, P = 0.005) for IL-10, and 0.554 (0.365–0.743, P = 0.588) for IL-6. In addition, the best cut-off values, and the sensitivity and specificity for each value were: 722 pg/mL, 92%, and 97% for presepsin; 3,530 μg/L, 100%, and 87% for ferritin; 1,715 U/mL, 100%, and 84% for sIL-2R; 55 pg/mL, 33%, and 87% for IFN-γ; 5.3 pg/mL, 100%, and 48% for IL-10; and 38 pg/mL, 50%, and 68% for IL-6, respectively.

Based on the ROC analyses, the diagnostic performance of plasma presepsin for HPS was found to be excellent (AUC of 0.934), and almost similar to that of serum ferritin (0.981) or sIL-2R (0.954). From the viewpoint of specificity, presepsin was the best diagnostic marker, whereas ferritin and sIL-2R were the best with regard to sensitivity. These findings suggest that a composite model of presepsin and ferritin/sIL-2R might have an increased diagnostic utility for HPS compared to each individual biomarker.

Allen et al. demonstrated the excellent diagnostic utility of hyperferritinemia >10,000 μg/L, which has a sensitivity of 90% and a specificity of 96%, for HPS using retrospective analyses [4]. In our data as well, serum ferritin had a sensitivity of 100% and a specificity of 87% for HPS when using a cut-off value of 3,530 μg/L by the Youden index. Xu et al. reported that with an IFN-γ level of >100 pg/mL, the sensitivity was 94.4% and the specificity was 98.9 % for HPS; and when the IFN-γ level was >75 pg/mL and that of IL-10 was >60 pg/mL, the sensitivity was 93.0% and specificity was 97.2% for HPS, in study subjects about half of whom had infection-related HPS and about 40% of whom had HPS of unknown etiology [7]. These data, especially for IFN-γ, were inconsistent with our findings. Although we have not definitively identified the reason for this discrepancy, differences in the study populations, including variations in the causes of HPS, may have influenced the results.

Presepsin is a 13-kDa protein and is thought to be released as fragment of CD14, mainly from monocytes, in response to engulfment of bacteria and also of monosodium urate crystals [9]. Increasing evidence indicates that presepsin is useful as a diagnostic and prognostic marker for sepsis [8]. Recent reports suggest that blood levels of presepsin are elevated in patients with HPS, and these alterations might be useful for predicting the prognosis of HPS [9,10]. However, to our knowledge, our study is the first to evaluate the diagnostic value of presepsin for HPS. Note that elevated levels of ferritin in HPS may be involved in growth

differentiation factor 15-mediated upregulation of ferroportin, as well as in its production by activated macrophages [1,14]; also that sIL-2R may reflect activation of T cells [15]. Thus, presepsin, which more directly reflects the activation of macrophages, could have different diagnostic potentials for HPS. Furthermore, from the viewpoint of timely availability, since it may be difficult to measure sIL-2R quickly in clinical practice, a composite model of presepsin and ferritin, or a scoring system incorporating presepsin into the HScore [3] as a diagnostic tool for secondary HPS, may warrant further investigation.

Our study has several limitations: (i) Selecting appropriate study controls was a challenge. Focusing on fever, cytopenias and hyperferritinemia, which are included in the HLH-2004 criteria [2], we chose control patients who had FN and serum ferritin levels of >500 μg/L. Our best cut-off data for ferritin was 3,530 μg/L, which is much lower than the 10,000 μg/L reported by Allen [4]. This may be because of differences in the control populations. Therefore, the results of our study should be interpreted with care, especially with regard to the cut-off values. However, because we measured each biomarker at the same time in the same samples, which were collected at the same time, our study comparisons are the most objective, a major strength of our study. (ii) The causes of HPS included predominantly infections and allogeneic stem cell transplantation cases. Therefore, our results may not apply

to other etiologies, such as autoimmune-related HPS.

In conclusion, we have demonstrated that plasma presepsin could be a useful diagnostic biomarker for HPS, and that it has high specificity.

Additional studies are needed to assess the usefulness of diagnostic tools including plasma presepsin.

Potential conflict of interest

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Figure Legends

Figure 1. Receiver operating characteristic curves for plasma presepsin, serum ferritin, sIL-2R, IFN-γ, IL-10 and IL-6 for the diagnosis of hemophagocytic syndrome

Table 1. Background characteristics of control febrile neutropenia (FN) cases and patients with hemophagocytic syndrome (HPS), and distributions of each biomarker at onset of FN and HPS

	Control (n = 22)	HPS (n = 12)
Age, years	47 (20–70)	47 (22–65)
Male sex	10 (45.5)	7 (58.3)
Primary disease		
Acute leukemia	15 (68.2)	7 (58.3)
Myelodysplastic syndrome	2 (9.1)	3 (25.0)
Malignant lymphoma	4 (18.2)	2 (16.7)
Chronic myeloid leukemia	1 (4.5)	0 (0)
Details of FN episodes (n = 31) and HPS cases		
White blood cell count at onset (/μL)	200 (0–2,000)	200 (0–15,900)
Neutrophil count at onset (/μL)	0 (0–936)	0 (0–14,151)*
Treatment		
Chemotherapy	31 (100)	3 (25.0)
Allogeneic hematopoietic stem cell transplantation (Allo-HCT)	0 (0.0)	9 (75.0)
Before engraftment		7
After engraftment		2
Causes of FN		
Sepsis	4 (12.9)	–
Local infection	8 (25.8)	–
Fever of unknown origin	16 (51.6)	–
Drug fever	3 (9.7)	–
Causes of HPS		

Infection†	–	7 (58.3)	
Lymphoma	–	1 (8.3)	
Allo-HCT	–	3 (25.0)	
Drug‡	–	1 (8.3)	
HPS diagnostic criteria§			
Fever	–	12/12 (100)	
Splenomegaly	–	12/12 (100)	
Hyperferritinemia ≥500 μg/L	–	12/12 (100)	
Hypertrigleceridemia ≥265 mg/dL or hypofibrinogenemia ≤1.5 g/L	–	2/12 (17)	
Histopathological findings of hemophagocytosis	–	12/12 (100)	
Cytopenias	–	5/5 (100)	
High sIL-2R ≥2,400 U/mL	–	3/4 (75)	
Low or absent natural killer cell activity	–	1/1 (100)	
Distributions of each biomarker at onset of FN and HPS			
Plasma presepsin levels (pg/mL)	225 (115–876)	1,935 (182–11,800)	‖$P < 0.0001$
Serum ferritin levels (μg/L)	905 (530–5,420)	25,700 (3,580–926,000)	‖$P < 0.0001$
Serum sIL-2R levels (U/mL)	843 (277–5,870)	4,585 (1,830–68,900)	‖$P < 0.0001$
Serum IFN-γ levels (pg/mL)	22 (0–81)	27 (0–1,252)	‖$P = 0.509$
Serum IL-10 levels (pg/mL)	5.8 (0.0–202)	23 (5.6–174)	‖$P = 0.004$
Serum IL-6 levels (pg/mL)	22 (0.5–436)	34 (7.1–224)	‖$P = 0.601$

Data are shown as median (range) or number (percentage) of subjects.

*Two cases were not included due to missing information.

†A total of seven infection cases were included as follows: one with candidemia, one with *Aspergillus* pneumonia, one with *Hormographiella aspergillata* pneumonia, one with both *Aspergillus* pneumonia and BK virus cystitis, one with BK virus cystitis, one with *Clostridium difficile* enteritis, and one with urinary tract infection.

‡Drug indicated cephem antibiotic.

§A total of seven pre-engraftment HPS cases were diagnosed by modified HPS criteria (reference 11), and the remaining five were diagnosed by standard HLH-2004 criteria. Of the five cases, regarding sIL-2R and natural killer cell activity, data were available in four and one, respectively.

||All *P* values were calculated by Mann-Whitney test.

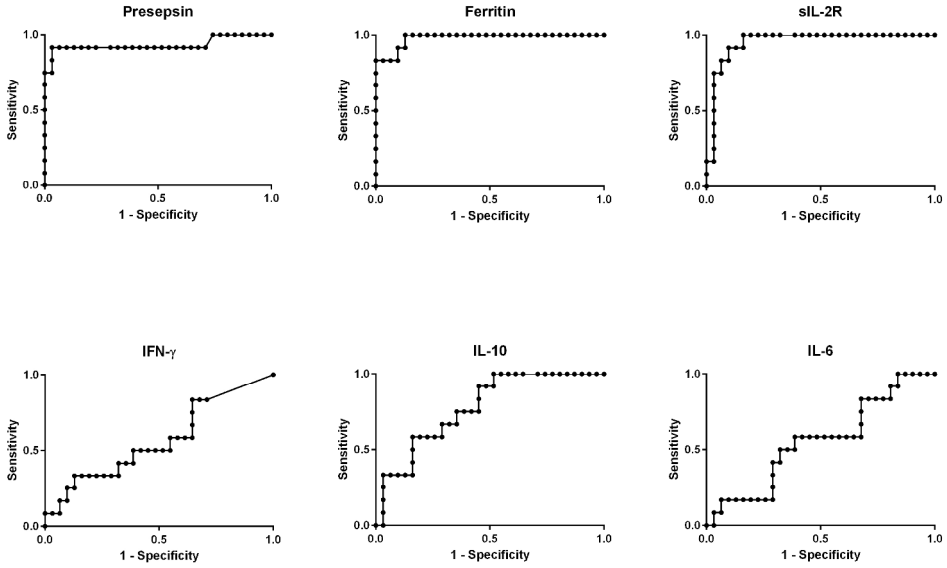

Figure 1

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