INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Plasma Levels of Presepsin (Soluble CD14-subtype) as a Novel Prognostic Marker for Hemophagocytic Syndrome in Hematological Malignancies

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Abstract

Objective Recent studies suggest that presepsin (soluble CD14-subtype) is a useful diagnostic and prognostic marker for sepsis, with secretion by activated macrophages potentially dependent on phagocytosis of microorganisms. As "hemophagocytosis" is one of the major characteristics in patients with hemophagocytic syndrome (HPS), we hypothesized that presepsin may reflect the phagocytic activity and be a useful prognostic marker for HPS. Therefore, we aimed to assess the prognostic potential of presepsin in secondary HPS in adult patients with hematological malignancies.

Methods Between April 2006 and August 2014, we retrospectively examined consecutive patients with HPS whose blood samples were available at our institution and compared the prognostic value of the following in HPS, singly and in combination: plasma presepsin, serum soluble interleukin (IL)-2 receptor (sIL-2R), ferritin, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-6 and IL-10.

Results A total of 14 patients were enrolled. The median age of the patients was 46.5 years (range, 22-65). In univariable Cox models, there were no significant variables associated with the prognosis. However, in 12 evaluable patients, only the combination of higher median values of presepsin (>1,935 pg/mL) and sIL-2R (>4,585 U/mL) at the onset of HPS was significantly associated with the 90-day mortality (hazard ratio 14.5; 95% CI, 1.47-143.36; p=0.02).

Conclusion These results suggest that a composite model of plasma presepsin and serum sIL-2R levels at the onset of HPS might be a novel predictor of the prognosis of patients with hematological malignancies and secondary HPS.

Key words: hemophagocytic syndrome, prognostic factor, presepsin (soluble CD14-subtype), soluble interleukin-2 receptor

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Introduction

Hemophagocytic syndrome (HPS) is a rare, potentially fatal cytokine-related disorder, characterized mainly by a high fever, cytopenias, splenomegaly and hemophagocytosis as pathological findings (1). HPS is classified into two categories: a familial form caused by genetic factors presenting at a young age and a secondary form triggered by infections, malignancies, autoimmune diseases and transplantation in adults (2, 3). The pathophysiology is not fully understood, but has been explained mainly by the fact that the impaired

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cytotoxic function of natural killer (NK) cells and cytotoxic lymphocytes, together with abnormal activation of macrophages, cause dysregulated and persistent immune stimulation, excessive cytokine production by these cells and a hyperinflammatory state (4-6).

Secondary HPS in adults is due to a heterogeneous group of underlying causes, as described above (2, 3). Generally, in secondary HPS, the underlying causes should be treated. In refractory cases, immunosuppressive agents such as steroids and calcineurin inhibitors (CI) and etoposide are used according to the HLH-2004 protocol. Despite this treatment, the mortality is reportedly high at 20-75% (7-9). Several previous studies showed that the following clinical and laboratory findings were risk factors associated with a very poor prognosis: underlying malignant lymphoma, increasing age, a low platelet count, management without etoposide (8), low levels of albumin (9) and low levels of fibrinogen (10). Other previous studies reported that immune-related biomarkers such as soluble interleukin-2 receptor (sIL-2R) (11), ferritin (12, 13), tumor necrosis factor-α (TNF-α) (14), interferon-γ (IFN-γ), IL-6 (15) and IL-10 (16) may be associated with the prognosis in patients with HPS. However, the comparative superiority of these immune markers as predictors of the prognosis in HPS has not been clearly shown.

Presepsin (soluble CD14-subtype) has recently been reported to be a novel diagnostic and prognostic marker for sepsis (17, 18). Presepsin is a 13-kDa protein that is a truncated N-terminal fragment of CD14, a glycoprotein highly expressed on the membrane surface of monocytes and macrophages (19, 20). Although the biological function of presepsin remains unknown, according to the data from an experimental rabbit model (21), the release mechanism is thought to be associated with phagocytosis and cleavage of microorganisms by lysosomes. Quite recently, it was reported that presepsin secretion by human monocytes is triggered by a phagocytic stimulus, and serum presepsin levels were significantly elevated in patients with HPS after allogeneic hematopoietic cell transplantation (allo-HCT) (22). A phenomenon wherein activated macrophages engulf blood cells is commonly observed in patients with HPS. We therefore hypothesized that presepsin may reflect the phagocytic activity and be a more useful prognostic marker for HPS than previously reported markers.

In the present study, we sought to examine 1) whether the plasma level of presepsin was a superior prognostic marker for HPS compared with the serum levels of sIL-2R, ferritin, IFN-γ, TNF-α, IL-6 and IL-10 and 2) which combination of these markers was the most useful predictor of the survival and/or treatment response when compared to other immune-related markers on their own.

Materials and Methods

Patients

We retrospectively evaluated consecutive patients who developed HPS and whose blood samples were available at our institution between April 2006 and August 2014. Because it was difficult to obtain informed consent due to the retrospective nature of our study, we made a public announcement about the context of this study by posting a notice at our hospital and on our website in accordance with the ethical guidelines for epidemiological research compiled by both the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare in Japan. This study was reviewed and approved by the Human Subjects Review Committee at Osaka City University.

Definition of HPS

As a rule, we diagnosed HPS if a patient met at least five of the eight HLH-2004 criteria (1). The criteria are as follows: 1) a fever; 2) splenomegaly; 3) cytopenias; 4) hypertriglyceridemia and/or hypofibrinogenemia; 5) hemophagocytosis in the bone marrow, spleen, or lymph nodes; 6) decreased or absent NK activity; 7) a serum ferritin level ≥500 μg/L; and 8) a serum sIL-2R ≥2,400 U/mL. We were, however, unable to assess cytopenias and NK activity accurately when the peripheral blood cell count was poor due to the influence of chemotherapy, including conditioning regimens. In addition, it was considered that the serum levels of sIL-2R might be unreliable when diagnosing HPS in patients who had received allo-HCT, especially during the early phase, because it is well known that the serum levels of sIL-2R are substantially elevated in patients with active acute graft-versus-host-disease (aGVHD), and allogeneic immune responses likely cause an increase in these levels (23, 24). We therefore used the modified HLH-2004 criteria when diagnosing HPS before engraftment in allo-HCT patients, as follows: we diagnosed HPS when a patient showed pathological findings of hemophagocytosis and fulfilled at least three of the following four criteria: 1) a fever; 2) splenomegaly; 3) hypertriglyceridemia and/or hypofibrinogenemia; and 4) a serum ferritin level ≥500 μg/L. Furthermore, in order to assess underlying infections at the onset of HPS, we cultured samples obtained from suspected sites of infection and obtained imaging tests and fungal markers including plasma β-D glucan and serum *Aspergillus* galactomannan. In addition, we performed polymerase chain reaction analyses for adenovirus, herpes simplex virus, varicella zoster virus, Epstein-Barr virus, human herpes virus 6, cytomegalovirus, and parvovirus B19 in the peripheral blood with and without bone marrow.

Outcome measures

Outcome measures included the treatment response at

Table 1. Patient Characteristics at the Onset of Hemophagocytic Syndrome.

Characteristics	Number
n	14
Median age (range), years	46.5 (22–65)
Gender (male/female), n	9/5
Primary diseases, n (%)	
Acute myelogenous leukemia	5 (35.7)
Myelodysplastic syndrome	3 (21.4)
Acute lymphoblastic leukemia	2 (14.3)
Malignant lymphoma	4 (28.6)
Allogeneic hematopoietic cell transplantation, n (%)	10 (71.4)
Before engraftment	7 (50.0)
After engraftment	3 (21.4)
Documented Infections, n (%)	
Bacterial	2 (14.3)
Fungal*	4 (28.6)
Viral*	3 (21.4)
Causes of HPS, n (%)	
Infection	8 (57.1)
Transplantation	3 (21.4)
Lymphoma	2 (14.3)
Drug	1 (7.1)
Treatment for hemophagocytic syndrome†, n (%)	
Steroid and/or calcineurin inhibitor	8 (57.1)
Steroid and calcineurin inhibitor, followed by re-transplantation	1 (7.1)
Etoposide plus steroid and/or calcineurin inhibitor	5 (35.7)
Follow up time from onset (range), days	173.5 (11–1,185)

* One patient had both viral and fungal infection at the onset.
† Treatment was administered between the date of diagnosis and day 90 after the diagnosis.

eight weeks and mortality at 90 days after the onset of HPS. We used the treatment response criteria as described previously (25). A complete response (CR) was defined as both resolution of the clinical symptoms and normalization of the laboratory findings. A partial response (PR) was defined as an improvement in the clinical symptoms, laboratory findings or both, despite a CR not being achieved. The progression of disease/relapse (PD) was defined as aggravation of the clinical symptoms, laboratory findings or both.

Presepsin and cytokine analyses in the peripheral blood

Peripheral blood samples were collected before and during the course of HPS. Blood samples were separated by centrifuging the blood at 3,000 rpm for 20 minutes and then stored at -80℃ until they were analyzed. The plasma levels of presepsin were measured by the PATHFAST Presepsin kit (LSI Medience Corporation, Tokyo, Japan). The serum levels of sIL-2R and ferritin were evaluated using the Siemens Immulyze IL-2R II kit and Chemilumi ACS-Ferritin II kit (Siemens Healthcare Diagnostics, Tokyo, Japan), respectively. The serum levels of IFN-γ, TNF-α, IL-6 and IL-10 were determined by the Bio-Plex Pro Cytokine Assay system (Bio-Rad Laboratories, Hercules, USA).

Statistical analysis

The time points for blood sample collection were defined as below: 1) baseline, before the onset of HPS; 2) onset, immediately after the onset of HPS within 5 days of the diagnosis; 3) peak, the time at which the level of each marker was highest during the course of HPS, and 4) active phase, during the course of HPS. We employed the Wilcoxon signed-rank test to compare the peripheral blood levels of immune-related markers between baseline and at the onset of HPS, and at baseline and at the peak phase. Spearman's rank correlation coefficient was calculated to assess the relationship between two variables. Cox proportional hazards models were used to investigate the risk factors for treatment response at eight weeks after the onset of HPS and mortality at 90 days after the onset of HPS. The proportional hazards assumption was checked for all continuous variables by a log-minus-log plot.

All p-values and 95% confidence intervals were two-tailed. A value of $p<0.05$ was considered to be statistically significant. Statistical analyses were performed using the SPSS statistics version 22.0 (SPSS, Chicago, USA) and GraphPad Prism version 5.0 software programs (GraphPad Software Inc., San Diego, USA).

Results

A total of 14 patients with HPS were eligible and evaluable. A total of 61 samples were available: 14 baseline samples before the onset of HPS in eight patients; 12 samples at the onset in 12 patients; and 35 samples after the onset in 13 patients. The patient characteristics are shown in Table 1. The median age was 46.5 years (range, 22-65 years). The underlying diseases were predominantly acute leukemia and myelodysplastic syndrome. Ten patients had undergone allo-HCT. Of these patients, seven patients developed HPS be-

Table 2. Clinical Features and Outcomes of Patients with New-onset Secondary Hemophagocytic Syndrome (HPS).

Patient No.	Age (years) /sex	Primary disease /status	Causes of HPS	Treatments for HPS*	Treatment response for HPS at eight weeks	Follow-up time from the onset of HPS (days)	Outcomes (cause of death)
Patients with active malignancy							
1	58/F	ML /active	Lymphoma	Steroid	PD	47	Dead (primary disease)
2	31/M	ML /active	Lymphoma	Etoposide and steroid	PD	74	Dead (primary disease)
3	65/M	AML /active	Infection (*Asperugillus* pneumonia)	Steroid	PD	299	Dead (GVHD with infection)
4	45/M	AML /active	Drug (Cephem allergy suspected)	Etoposide and steroid	PR	343	Alive
Patients who developed HPS at pre-engraftment phase after allogeneic hematopoietic stem cell transplantation							
5	44/F	AML /CR	Infection (*Clostridium difficile* enteritis)	Steroid and CI	PR	1075	Alive
6	53/F	MDS /active	Infection (BK virus cystitis)	Steroid and CI	PD	11	Dead (interstitial pneumonia)
7	34/F	MDS /active	Infection (BK virus cystitis and *Aspergillus* pneumonia)	Steroid and CI	PD	43	Dead (GVHD with infection)
8	51/M	MDS /active	Infection (*Hormographiella aspergillata* pneumonia)	Steroid and CI, followed by re-transplantation	PD	43	Dead (engraftment failure with infection)
9	48/M	AML /CR	Transplantation	Steroid and CI	PR	1185	Alive
10	22/M	ALL /CR	Transplantation	Etoposide and CI	PR	126	Dead (relapse of primary disease)
11	64/M	ML /active	Transplantation	Etoposide plus steroid and CI	PR	200	Dead (relapse of primary disease)
Patients who developed HPS at post-engraftment phase after allogeneic hematopoietic stem cell transplantation							
12	32/M	AML /active	Infection (Candidemia)	CI	PR	614	Dead (relapse of primary disease with infection)
13	40/F	ALL /CR	Infection (Urinary tract infection)	Steroid	PR	724	Alive
14	55/M	ML /CR	Infection (CMV viremia)	Etoposide plus steroid and CI	PD	147	Dead (GVHD with infection)

F: female, M: male, ML: malignant lymphoma, AML: acute myelogenous leukemia, MDS: myelodysplastic syndrome, ALL: acute lymphoblastic leukemia, CMV: cytomegalovirus, CI: calcineurin inhibitor, PR: partial response, PD: progression of disease/relapse, GVHD: graft-versus-host disease

*Treatment was initiated between the date of diagnosis and day 90 after the diagnosis.

fore engraftment. The triggers of HPS included eight infections, three allo-HCTs, two lymphomas and one drug-related cause. In addition, eight patients were treated with steroids and/or CI, one with steroids and CI followed by re-transplantation, and five with etoposide plus steroids and/or CI. The median follow-up time in all patients and in the four survivors was 174 days (range, 11-1,185) and 900 days (343-1,185), respectively.

Treatment response at eight weeks and the outcomes

The clinical features and outcomes of patients with HPS are summarized in Table 2. No patient was able to achieve a CR at eight weeks after the onset of HPS. Seven patients were able to attain a PR, while seven patients developed PD within eight weeks. Of 14 patients, only four patients survived to the final follow-up. The causes of death during the follow-up were the primary disease in four patients, GVHD with infection in three patients, progression of the primary disease with infection in one patient, engraftment failure with infection in one patient, and interstitial pneumonia in one patient.

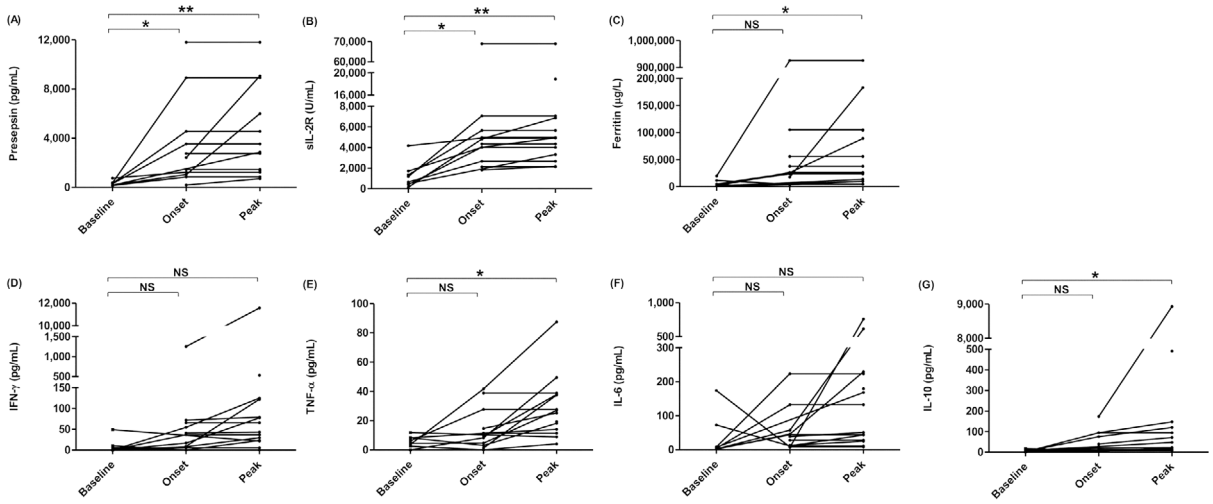

Figure. **Comparison of the levels of plasma presepsin and serum immune-related markers at baseline (n=8; patients No. 4-9, 12, 14), at onset (n=12; patients No. 1, 3-13) and in the peak phase (n=14) in patients with hemophagocytic syndrome. (A) Presepsin; (B) sIL-2R; (C) Ferritin; (D) IFN-γ; (E) TNF-α; (F) IL-6; and (G) IL-10. sIL-2R: soluble interleukin-2 receptor, IFN-γ: interferon-γ, TNF-α: tumor necrosis factor-α, IL: interleukin, NS: not statistically significant. *p value<0.05, **p value<0.01**

Kinetics of the plasma levels of presepsin and serum levels of sIL-2R, ferritin and cytokines during the follow-up of HPS

In eight patients who had samples at baseline (patients No. 4-9, 12, 14), the median levels of presepsin, sIL-2R, ferritin, IFN-γ, TNF-α, IL-6 and IL-10 were 241.0 (range, 144-755) pg/mL, 921.5 (144-4,180) U/mL, 3,225 (1,180-19,900) μg/L, 2.03 (0.00-49.33) pg/mL, 5.33 (0.00-11.91) pg/mL, 5.44 (1.79-174.4) pg/mL and 5.32 (1.42-17.62) pg/mL, respectively. In 12 patients who had samples at the onset (patients No. 1, 3-13), the median levels of presepsin, sIL-2R, ferritin, IFN-γ, TNF-α, IL-6 and IL-10 at the onset were 1,935 (range, 182-11,800) pg/mL, 4,585 (1,830-68,900) U/mL, 25,700 (3,580-926,000) μg/L, 26.92 (0.0-1,252) pg/mL, 10.43 (0.01-41.73) pg/mL, 33.58 (7.1-223.5) pg/mL and 23.27 (5.59-173.7) pg/mL, respectively. In all 14 patients, the median levels of presepsin, sIL-2R, ferritin, IFN-γ, TNF-α, IL-6 and IL-10 at the peak phase were 2,805 (range, 705-11,800) pg/mL, 4,930 (2,130-68,900) U/mL, 32,200 (4,530-926,000) μg/L, 54.8 (0.00-11,573) pg/mL, 26.17 (4.12-87.51) pg/mL, 91.78 (8.07-758.8) pg/mL and 35.29 (8.05-8,931) pg/mL, respectively.

In seven patients who had samples both at baseline and at the onset (patients No. 4-9, 12), both the median levels of plasma presepsin (p=0.02) and serum sIL-2R (p=0.02) were significantly elevated at the onset when compared with the baseline (Figure A and B). In contrast, there were no significant differences between the baseline levels and median levels at the onset for ferritin (p=0.08), IFN-γ (p=0.22), TNF-α (p=0.22), IL-6 (p=0.58) and IL-10 (p=0.11) (Figure C-G). In eight patients who had samples both at baseline and at the peak phase, the median peak levels of presepsin (p<0.01), sIL-2R (p<0.01), ferritin (p=0.02), TNF-α (p=0.02) and IL-10 (p=0.04), but not IFN-γ (p=0.11) or IL-6 (p=0.15), were significantly higher than those at baseline (Figure A-G).

Correlations among the levels of plasma presepsin and serum immune-related markers in patients with HPS at the onset

We assessed the correlations among the levels of immune-related markers in HPS at the onset (12 samples from 12 patients: patients No. 1, 3-13) and in the active phase (47 samples from 14 patients) (Table 3). In addition, we evaluated the correlations between these immune-related markers and the peripheral blood cell numbers of neutrophils, lymphocytes and monocytes according to available data. Because white blood cell counts were low due to the conditioning regimens of allo-HCT, the correlations between the immune-related markers and the peripheral blood cell numbers at the onset of HPS were evaluable in just eight patients (patients No. 1, 3, 4, 6, 7, 9, 12, 13); those in the active phase were evaluable in 37 samples from 14 patients.

There was a significant correlation between the serum ferritin and serum TNF-α levels at the onset (r=0.66, p=0.020). Apart from this combination, there were no significant correlations among the immune-related markers at the onset (Table 3A). In contrast, there were many combinations that showed significant correlations during the active phase of HPS (Table 3B). In addition, we found a significant positive correlation between the numbers of monocytes and neutrophils (r=0.86, p=0.007; r=0.80, p<0.001, respectively), the numbers of monocytes and lymphocytes (r=0.78, p=0.022; r=0.56, p<0.001, respectively) and the numbers of neutrophils and lymphocytes (r=0.80, p=0.015; r=0.33, p=0.049, respectively) at the onset and during the active phase of

Table 3.

(A) Spearman Correlation Coefficients among Plasma Presepsin Levels and Serum Immune-related Markers at the Onset of Hemophagocytic Syndrome (n=12).

	sIL-2R		Ferritin		IFN-γ		TNF-α		IL-6		IL-10	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
Presepsin	0.42	0.175	0.27	0.404	0.27	0.403	0.26	0.417	-0.12	0.713	-0.13	0.697
sIL-2R			0.05	0.880	0.09	0.787	-0.16	0.618	-0.50	0.095	0.21	0.513
Ferritin					0.48	0.111	0.66	0.020	0.14	0.665	0.29	0.366
IFN-γ							0.50	0.100	-0.11	0.737	0.37	0.240
TNF-α									0.36	0.245	-0.05	0.880
IL-6											-0.11	0.729
IL-10												
Neutrophil*												
Lymphocyte*												

	Neutrophil*		Lymphocyte*		Monocyte*	
	r	p value	r	p value	r	p value
Presepsin	0.32	0.435	-0.06	0.888	0.18	0.670
sIL-2R	0.44	0.272	-0.13	0.756	0.16	0.713
Ferritin	-0.16	0.713	-0.16	0.713	-0.25	0.548
IFN-γ	0.43	0.290	0.07	0.876	0.39	0.337
TNF-α	-0.34	0.417	-0.48	0.230	-0.31	0.453
IL-6	-0.76	0.031	-0.56	0.146	-0.61	0.108
IL-10	0.08	0.844	-0.13	0.756	0.16	0.713
Neutrophil*			0.80	0.015	0.86	0.007
Lymphocyte*					0.78	0.022

(B) Spearman Correlation Coefficients among Plasma Presepsin Levels and Serum Immune-related Markers in the Active Phase of Hemophagocytic Syndrome (n=47).

	sIL-2R		Ferritin		IFN-γ		TNF-α		IL-6		IL-10	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
Presepsin	0.35	0.015	0.38	0.009	0.23	0.11	0.13	0.402	0.46	0.001	0.05	0.733
sIL-2R			0.46	0.001	0.32	0.030	-0.08	0.595	0.09	0.550	0.58	<0.001
Ferritin					0.52	<0.001	0.31	0.037	0.38	0.008	0.49	0.001
IFN-γ							0.66	<0.001	0.34	0.020	0.58	<0.001
TNF-α									0.49	<0.001	0.24	0.103
IL-6											0.15	0.326
IL-10												
Neutrophil†												
Lymphocyte†												

	Neutrophil†		Lymphocyte†		Monocyte†	
	r	p value	r	p value	r	p value
Presepsin	0.14	0.395	0.07	0.681	-0.05	0.793
sIL-2R	-0.10	0.576	-0.23	0.164	-0.35	0.032
Ferritin	0.01	0.972	-0.06	0.739	-0.23	0.174
IFN-γ	0.09	0.579	-0.01	0.977	-0.10	0.561
TNF-α	-0.09	0.608	0.10	0.544	-0.05	0.770
IL-6	-0.06	0.745	-0.07	0.697	-0.17	0.329
IL-10	-0.06	0.712	-0.14	0.394	-0.21	0.214
Neutrophil†			0.33	0.049	0.80	<0.001
Lymphocyte†					0.56	<0.001

sIL-2R: soluble interleukin-2 receptor, IFN-γ: interferon-γ, TNF-α: tumor necrosis factor-α, IL: interleukin, r: correlation coefficient calculated using Spearman's method.

*Of twelve patients who had samples at the onset of HPS, eight patients were evaluable (patient No. 1, 3, 4, 6, 7, 9, 12, 13). The remaining four patients were excluded because we were unable to measure accurately cell numbers of monocytes, neutrophils and lymphocytes in peripheral blood due to extremely low blood cell counts.

†A total of 37 samples from 14 patients were analyzed. The remaining four samples at the onset and six samples after the onset from five patients (patient No. 3, 5, 8, 10, 11) were excluded due to extremely low blood cell counts.

HPS. Furthermore, we found a significant negative correlation between the numbers of neutrophils and levels of IL-6 at the onset of HPS (r=-0.76, p=0.031) and a significant negative correlation between the numbers of monocytes and levels of sIL-2R during the active phase of HPS (r=-0.35, p=0.032).

The 90-day mortality and the prognostic factors in HPS

In 12 patients who had samples at the onset (patients No.

Table 4. Comparison of Immune-related Biomarkers and Their Combinations at the Onset of Hemophagocytic Syndrome in Predicting Mortality at Day 90.

Model	Hazard Ratio (95% CI)	p value
Continuous univariable analysis		
Presepsin, per 1 s.d.	1.31 (0.57–3.04)	0.526
sIL-2R, per 1 s.d.	1.43 (0.75–2.72)	0.280
Ferritin, per 1 s.d.	1.55 (0.76–3.17)	0.229
IFN-γ, per 1 s.d.	1.40 (0.72–2.70)	0.320
TNF-α, per 1 s.d.	1.13 (0.43–2.99)	0.806
IL-6, per 1 s.d.	0.53 (0.09–3.10)	0.479
IL-10, per 1 s.d.	1.51 (0.67–3.45)	0.322
Categorical univariable analysis		
Presepsin, pg/mL		
Median 1 (≤1,935)	1.00 (reference)	
Median 2 (>1,935)	3.48 (0.36–33.52)	0.280
sIL-2R, U/mL		
Median 1 (≤4,585)	1.00 (reference)	
Median 2 (>4,585)	3.48 (0.36–33.52)	0.280
Ferritin, μg/L		
Median 1 (≤25,700)	1.00 (reference)	
Median 2 (>25,700)	0.29 (0.03–2.77)	0.280
IFN-γ, pg/mL		
Median 1 (≤26.92)	1.00 (reference)	
Median 2 (>26.92)	0.86 (0.12–6.14)	0.883
TNF-α, pg/mL		
Median 1 (≤10.43)	1.00 (reference)	
Median 2 (>10.43)	1.16 (0.16–8.24)	0.883
IL-6, pg/mL		
Median 1 (≤33.58)	1.00 (reference)	
Median 2 (>33.58)	0.97 (0.14–6.90)	0.972
IL-10, pg/mL		
Median 1 (≤23.27)	1.00 (reference)	
Median 2 (>23.27)	2.92 (0.30–28.20)	0.355

(continue)

1, 3-13), we evaluated whether individual immune-related biomarkers and their combinations could predict the 90-day mortality after the onset of HPS. Of the 12 patients, eight patients survived to day 90 after the onset.

In the univariable Cox models, there was no significant relationship between any immune-related marker and the 90-day mortality.

In the multivariable Cox models, only the combination of presepsin (>1,935 pg/mL) and sIL-2R (>4,585 U/mL) was significantly associated with the 90-day mortality (hazard ratio 14.5, 95% CI, 1.47-143.36; p=0.02), whereas the other combinations did not achieve statistical significance (Table 4).

Treatment response at 8 weeks and the predictive factors for its response

In 12 patients who had samples at the onset (patients No. 1, 3-13), we analyzed the predictive factors for a treatment response eight weeks after the onset. Seven patients attained a PR and five patients had PD at eight weeks.

In the univariable Cox models, there were no significant associations between each immune-related marker and the treatment response at eight weeks.

In the multivariable Cox models, only the combination of presepsin (>1,935 pg/mL) and sIL-2R (>4,585 U/mL) was significantly associated with a treatment response at eight weeks (hazard ratio 14.5, 95% CI, 1.47-143.36; p=0.02), whereas the other combinations were not significant (Table 5).

Discussion

In the present study, we found that the combination of plasma presepsin and serum sIL-2R was the most useful predictor of the treatment response and mortality in patients with hematological malignancies and secondary HPS, compared with the other combinations of immune-related markers. No single marker was associated with the prognosis in our study.

Previous reports showed that several immune-related markers were associated with a poor prognosis in HPS. A serum sIL-2R level >10,000 U/mL at the peak of HPS mainly in children (11), serum IL-10 ≥2,000 pg/mL at the peak of HPS in children (16), serum IL-6 >300 pg/mL and serum IFN-γ >30 U/mL or serum sIL-2R >10,000 U/mL at the peak of HPS mainly in children (15), serum TNF-α ≥50 pg/mL at the onset of HPS in children (14), serum ferritin >11,000 μg/L at the peak of HPS within three weeks after the onset and less than 50% decrease in serum ferritin between the day when the patients developed HPS and the day when the ferritin levels were the lowest during 0-10 weeks after the onset in children (12), and serum ferritin >50,000

Table 4. continued

Model	Hazard Ratio (95% CI)	p value
Composite model analysis		
Presepsin, pg/mL and sIL-2R, U/mL		
Group 1 (Presepsin≤1,935 and/or sIL-2R≤4,585)	1.00 (reference)	
Group 2 (Presepsin>1,935 and sIL-2R>4,585)	14.51 (1.47–143.36)	0.022
Presepsin, pg/mL and Ferritin, μg/L		
Group 1 (Presepsin≤1,935 and/or Ferritin≤25,700)	1.00 (reference)	
Group 2 (Presepsin>1,935 and Ferritin>25,700)	0.98 (0.10–9.41)	0.984
Presepsin, pg/mL and IFN-γ, pg/mL		
Group 1 (Presepsin≤1,935 and/or IFN-γ≤26.92)	1.00 (reference)	
Group 2 (Presepsin>1,935 and IFN-γ>26.92)	1.87 (0.26–13.30)	0.531
Presepsin, pg/mL and TNF-α, pg/mL		
Group 1 (Presepsin≤1,935 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (Presepsin>1,935 and TNF-α>10.43)	2.66 (0.37–19.04)	0.331
Presepsin, pg/mL and IL-6, pg/mL		
Group 1 (Presepsin≤1,935 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (Presepsin>1,935 and IL-6>33.58)	0.98 (0.10–9.41)	0.984
Presepsin, pg/mL and IL-10, pg/mL		
Group 1 (Presepsin≤1,935 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (Presepsin>1,935 and IL-10>23.27)	5.33 (0.74–38.35)	0.097
sIL-2R, U/mL and Ferritin, μg/L		
Group 1 (sIL-2R≤4,585 and/or Ferritin≤25,700)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and Ferritin>25,700)	0.98 (0.10–9.41)	0.984
sIL-2R, U/mL and IFN-γ, pg/mL		
Group 1 (sIL-2R≤4,585 and/or IFN-γ≤26.92)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and IFN-γ>26.92)	2.94 (0.41–20.93)	0.282
sIL-2R, U/mL and TNF-α, pg/mL		
Group 1 (sIL-2R≤4,585 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and TNF-α>10.43)	4.69 (0.64–34.28)	0.128
sIL-2R, U/mL and IL-6, pg/mL		
Group 1 (sIL-2R≤4,585 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and IL-6>33.58)	1.75 (0.18–17.03)	0.628
sIL-2R, U/mL and IL-10, pg/mL		
Group 1 (sIL-2R≤4,585 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (sIL2R>4,585 and IL-10>23.27)	1.87 (0.26–13.30)	0.531
Ferritin, μg/L and IFN-γ, pg/mL		
Group 1 (Ferritin≤25,700 and/or IFN-γ≤26.92)	1.00 (reference)	
Group 2 (Ferritin>25,700 and IFN-γ>26.92)	0.62 (0.07–5.99)	0.682
Ferritin, μg/L and TNF-α, pg/mL		
Group 1 (Ferritin≤25,700 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (Ferritin>25,700 and TNF-α>10.43)	0.62 (0.07–5.99)	0.682
Ferritin, μg/L and IL-6, pg/mL		
Group 1 (Ferritin≤25,700 and IL-6≤33.58)	1.00 (reference)	
Group 2 (Ferritin>25,700 and IL-6>33.58)	0.98 (0.10–9.41)	0.984
Ferritin, μg/L and IL-10, pg/mL		
Group 1 (Ferritin≤25,700 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (Ferritin>25,700 and IL-10>23.27)	0.62 (0.07–5.99)	0.682
IFN-γ, pg/mL and TNF-α, pg/mL		
Group 1 (IFN-γ≤26.92 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (IFN-γ>26.92 and TNF-α>10.43)	0.42 (0.04–4.04)	0.452
IFN-γ, pg/mL and IL-6, pg/mL		
Group 1 (IFN-γ≤26.92 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (IFN-γ>26.92 and IL-6>33.58)	0.98 (0.10–9.41)	0.984
IFN-γ, pg/mL and IL-10, pg/mL		
Group 1 (IFN-γ≤26.92 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (IFN-γ>26.92 and IL-10>23.27)	2.94 (0.41–20.93)	0.282
TNF-α, pg/mL and IL-6, pg/mL		
Group 1 (TNF-α≤10.43 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (TNF-α>10.43 and IL-6>33.58)	0.98 (0.10–9.41)	0.984
TNF-α, pg/mL and IL-10, pg/mL		
Group 1 (TNF-α≤10.43 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (TNF-α>10.43 and IL-10>23.27)	1.75 (0.18–17.03)	0.628
IL-6,pg/mL and IL-10, pg/mL		
Group 1 (IL-6≤33.58 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (IL-6>33.58 and IL-10>23.27)	3.58 (0.48–26.73)	0.213

95% CI: 95% confidence intervals, s.d.: standard deviation, sIL-2R: soluble interleukin-2 receptor, IFN-γ: interferon-γ, TNF-α: tumor necrosis factor-α, IL: interleukin

Table 5. Comparison of Immune-related Biomarkers and Their Combinations at the Onset of Hemophagocytic Syndrome in Predicting Treatment Response at Eight Weeks.

Model	Hazard Ratio (95% CI)	p value
Continuous univariable analysis		
Presepsin, per 1 s.d.	1.10 (0.48–2.54)	0.814
sIL-2R, per 1 s.d.	1.41 (0.74–2.71)	0.298
Ferritin, per 1 s.d.	1.54 (0.75–3.16)	0.239
IFN-γ, per 1 s.d.	1.39 (0.72–2.69)	0.332
TNF-α, per 1 s.d.	1.04 (0.42–2.59)	0.936
IL-6, per 1 s.d.	0.42 (0.06–2.77)	0.421
IL-10, per 1 s.d.	1.78 (0.82–3.82)	0.143
Categorical univariable analysis		
Presepsin, pg/mL		
Median 1 (≤1,935)	1.00 (reference)	
Median 2 (>1,935)	1.87 (0.31–11.28)	0.493
sIL-2R, U/mL		
Median 1 (≤4,585)	1.00 (reference)	
Median 2 (>4,585)	1.87 (0.31–11.28)	0.493
Ferritin, μg/L		
Median 1 (≤25,700)	1.00 (reference)	
Median 2 (>25,700)	0.53 (0.09–3.21)	0.493
IFN-γ, pg/mL		
Median 1 (≤26.92)	1.00 (reference)	
Median 2 (>26.92)	0.59 (0.10–3.55)	0.566
TNF-α, pg/mL		
Median 1 (≤10.43)	1.00 (reference)	
Median 2 (>10.43)	0.75 (0.13–4.50)	0.752
IL-6, pg/mL		
Median 1 (≤33.58)	1.00 (reference)	
Median 2 (>33.58)	0.65 (0.11–3.90)	0.634
IL-10, pg/mL		
Median 1 (≤23.27)	1.00 (reference)	
Median 2 (>23.27)	4.40 (0.49–39.87)	0.188

(continue)

μg/L at the onset of HPS in adults (13) were reported to be prognostic factors.

In our kinetic analysis (Figure), only presepsin and sIL-2R showed significant increases with the onset of HPS. Furthermore, we demonstrated that the combination of presepsin and sIL-2R at the onset was the strongest predictor of the prognosis in HPS (Table 4, 5). However, we did not identify why the other markers were not associated with the prognosis of HPS. These non-significant results might be explained by differences in the study population, including children or adults, and/or a low statistical power due to a small sample size.

In light of previous reports on the presepsin levels in patients with sepsis in the emergency department (17, 26), a level of 1,935 pg/mL at the onset of HPS was very high and appeared to reflect the clinical severity. Liu et al. showed that the median levels of plasma presepsin were 212, 325, 787 and 1,084 pg/mL in patients with systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis and septic shock, respectively. In this report, they also demonstrated that presepsin levels ≥556 pg/mL at the onset of sepsis were significantly associated with the 28-day mortality (17). Masson et al. reported that the median levels of plasma presepsin were 946 pg/mL in patients with severe sepsis including septic shock, and higher presepsin levels were associated with the ICU mortality and 90-day mortality (26). Consistent with our findings, Arai et al. recently reported that the serum presepsin level was elevated (approximately 3,000 pg/mL) in patients with HPS after allo-HCT (22).

In correlation analyses between the immune-related biomarkers and peripheral blood cell numbers, we found a significant negative correlation between the numbers of neutrophils and levels of IL-6 at the onset of HPS and a significant negative correlation between the numbers of monocytes and levels of sIL-2R during the active phase of HPS (Table 3). Although the precise explanations for these findings are unclear, certain reasons might be proposed. First, the negative correlation between the number of neutrophils and levels of IL-6 at the onset of HPS might be affected by the analytic population, which had infection-related HPS in four of the eight patients. More specifically, the serum levels of IL-6 may increase in some infections including those due to bacteria and fungi, and in addition to the low numbers of peripheral blood neutrophils, neutrophils might be consumed at the sites of infection, potentially leading to decreased numbers of neutrophils in the peripheral blood. Second, the negative correlation between the number of monocytes and levels of sIL-2R in the active phase of HPS might be explained as follows: elevated levels of sIL-2R in the serum may reflect activation of T cells, which is potentially related to activation of monocytes/macrophages in HPS; namely, ac-

Table 5. continued

Model	Hazard Ratio (95% CI)	p value
Composite model analysis		
Presepsin, pg/mL and sIL-2R, U/mL		
Group 1 (Presepsin≤1,935 and/or sIL2R≤4,585)	1.00 (reference)	
Group 2 (Presepsin>1,935 and sIL2R>4,585)	14.51 (1.47–143.36)	0.022
Presepsin, pg/mL and Ferritin, μg/L		
Group 1 (Presepsin≤1,935 and/or Ferritin≤25,700)	1.00 (reference)	
Group 2 (Presepsin>1,935 and Ferritin>25,700)	0.74 (0.08–6.59)	0.784
Presepsin, pg/mL and IFN-γ, pg/mL		
Group 1 (Presepsin≤1,935 and/or IFN-γ≤26.92)	1.00 (reference)	
Group 2 (Presepsin>1,935 and IFN-γ>26.92)	1.37 (0.23–8.23)	0.735
Presepsin, pg/mL and TNF-α, pg/mL		
Group 1 (Presepsin≤1,935 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (Presepsin>1,935 and TNF-α>10.43)	2.66 (0.37–19.04)	0.331
Presepsin, pg/mL and IL-6, pg/mL		
Group 1 (Presepsin≤1,935 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (Presepsin>1,935 and IL-6>33.58)	0.74 (0.08–6.59)	0.784
Presepsin, pg/mL and IL-10, pg/mL		
Group 1 (Presepsin≤1,935 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (Presepsin>1,935 and IL-10>23.27)	5.33 (0.74–38.35)	0.097
sIL-2R, U/mL and Ferritin, μg/L		
Group 1 (sIL-2R≤4,585 and/or Ferritin≤25,700)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and Ferritin>25,700)	0.74 (0.08–6.59)	0.784
sIL-2R, U/mL and IFN-γ, pg/mL		
Group 1 (sIL-2R≤4,585 and/or IFN-γ≤26.92)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and IFN-γ>26.92)	2.29 (0.37–14.06)	0.370
sIL-2R, U/mL and TNF-α, pg/mL		
Group 1 (sIL-2R≤4,585 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and TNF-α>10.43)	3.35 (0.55–20.59)	0.192
sIL-2R, U/mL and IL-6, pg/mL		
Group 1 (sIL-2R≤4,585 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and IL-6>33.58)	1.38 (0.15–12.50)	0.772
sIL-2R, U/mL and IL-10, pg/mL		
Group 1 (sIL-2R≤4,585 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (sIL-2R>4,585 and IL-10>23.27)	1.37 (0.23–8.23)	0.735
Ferritin, μg/L and IFN-γ, pg/mL		
Group 1 (Ferritin≤25,700 and/or IFN-γ≤26.92)	1.00 (reference)	
Group 2 (Ferritin>25,700 and IFN-γ>26.92)	0.46 (0.05–4.09)	0.483
Ferritin, μg/L and TNF-α, pg/mL		
Group 1 (Ferritin≤25,700 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (Ferritin>25,700 and TNF-α>10.43)	0.46 (0.05–4.09)	0.483
Ferritin, μg/L and IL-6, pg/mL		
Group 1 (Ferritin≤25,700 and IL-6≤33.58)	1.00 (reference)	
Group 2 (Ferritin>25,700 and IL-6>33.58)	0.74 (0.08–6.59)	0.784
Ferritin, μg/L and IL-10, pg/mL		
Group 1 (Ferritin≤25,700 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (Ferritin>25,700 and IL-10>23.27)	1.22 (0.20–7.30)	0.829
IFN-γ, pg/mL and TNF-α, pg/mL		
Group 1 (IFN-γ≤26.92 and/or TNF-α≤10.43)	1.00 (reference)	
Group 2 (IFN-γ>26.92 and TNF-α>10.43)	0.30 (0.03–2.69)	0.282
IFN-γ, pg/mL and IL-6, pg/mL		
Group 1 (IFN-γ≤26.92 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (IFN-γ>26.92 and IL-6>33.58)	0.74 (0.08–6.59)	0.784
IFN-γ, pg/mL and IL-10, pg/mL		
Group 1 (IFN-γ≤26.92 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (IFN-γ>26.92 and IL-10>23.27)	2.29 (0.37–14.06)	0.370
TNF-α, pg/mL and IL-6, pg/mL		
Group 1 (TNF-α≤10.43 and/or IL-6≤33.58)	1.00 (reference)	
Group 2 (TNF-α>10.43 and IL-6>33.58)	0.74 (0.08–6.59)	0.784
TNF-α, pg/mL and IL-10, pg/mL		
Group 1 (TNF-α≤10.43 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (TNF-α>10.43 and IL-10>23.27)	1.38 (0.15–12.50)	0.772
IL-6, pg/mL and IL-10, pg/mL		
Group 1 (IL-6≤33.58 and/or IL-10≤23.27)	1.00 (reference)	
Group 2 (IL-6>33.58 and IL-10>23.27)	2.68 (0.43–16.81)	0.294

95% CI: 95% confidence intervals, s.d.: standard deviation, sIL-2R: soluble interleukin-2 receptor, IFN-γ: interferon-γ, TNF-α: tumor necrosis factor-α, IL: interleukin

tivation or consumption of monocytes/macrophages in some target organs such as the liver or spleen might have resulted in decreased numbers of monocytes in the peripheral blood (27).

Abnormal continuous activation of macrophages as well as lymphocytes, including cytotoxic T and NK cells, may play a pivotal role in the pathophysiology of HPS (4-6). Presepsin is a newly discovered soluble form of CD14 on the surfaces of monocytes and macrophages (19), which is reported to be associated with phagocytosis (21, 22). Although the precise *in vivo* mechanism is unknown, Arai et al. indicated that elastase, a serine protease in human monocytes, mediated CD14 cleavage to produce presepsin after a phagocytic stimulus involving pathogens such as bacteria, as well as sterile agents such as monosodium urate crystals *in vitro* (22). In contrast, sIL-2R is a soluble form of the IL-2R α chain (CD25), which is a well-known marker for activated T cells *in vivo* and *in vitro* (28, 29). Therefore, plasma presepsin may reflect the extent of phagocytic activity of macrophages, whereas serum sIL-2R may reflect the extent of activation of T cells in patients. According to these observations, our data suggest that a combined model of plasma presepsin and serum sIL-2R, which could concurrently reflect the levels of activation of these cells, might be a powerful surrogate marker for the severity and prognosis of HPS. To the best of our knowledge, there are no other reports in which a disease or condition shows a simultaneous increase in the serum levels of both presepsin and sIL-2R. However, since it has been reported that the serum levels of sIL-2R were elevated in neonatal patients with sepsis and septic shock (30), it is possible that a simultaneous increase in the serum levels of presepsin and sIL-2R might be observed in these pathological conditions. Further research is needed to determine which disorders present with this type of dual increase and what it means.

There are some limitations associated with this study. The retrospective nature of the data, the small number of patients, the heterogeneity of the primary disease and triggers of HPS may limit the interpretation of our results. However, the major strengths of the present study are that the data for all seven biomarkers at each point were measured concurrently in the same sample and we compared all variables, including all combinations.

Taken together, these results provide evidence that a composite model of the plasma presepsin and serum sIL-2R levels at the onset of HPS, potentially a reflection of the activation of macrophages and T cells, respectively, might be a novel predictor of the severity and prognosis of HPS in adult patients with hematological malignancies. Further study is needed to validate the usefulness of this composite model.

Author's disclosure of potential Conflicts of Interest (COI).
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