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ORIGINAL

Early expression of serum CCL8 closely correlates to non-relapse mortality after allogeneic hematopoietic stem cell transplantation

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ABSTRACT

To explore the role of Chemokine (C-C motif) ligand 8 (CCL8) as a potential biomarker for acute graft-versus-host disease (aGVHD), we retrospectively analyzed the sera and clinical course of 31 patients with grade II–IV aGVHD. No deaths occurred in the ten patients with serum CCL8 concentrations less than 213 pg/mL, whereas 11 of the 21 patients with more than 213 pg/mL died within 180 days post-transplantation. This landmark analysis revealed a significantly lower survival rate of patients with a CCL8 serum concentration greater than 213 pg/mL. Thus, elevated serum CCL8 concentration before day 100 post-transplantation may predict aGVHD prognosis.

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Key words: GVHD, chemokine, CCL8, biomarker

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is widely used as a treatment for hematological and malignant diseases. However, despite the development of potent immunosuppressive agents, acute graft-versus-host disease (aGVHD) continues to be a major contributor to non-relapse mortality (NRM), such as infection, veno-occlusive disease and thrombotic microangiopathy (TAM), of patients treated with allogeneic HSCT for malignant diseases.[1, 2)]

Employing proteomic techniques, we have shown that CC chemokine-ligand motif 8 (CCL8) is a potential biomarker for the diagnosis of aGVHD.[3, 4)] We now report that elevated serum CCL8 concentration after HSCT closely correlates with NRM in humans. Thus, serum CCL8 levels might be a potential prognostic biomarker for the clinical outcome of grade II–IV aGVHD.

2. Patients and Methods

Thirty-one patients with grade II to IV aGVHD who underwent HSCT between May 1993 and February 2005 in the Department of Pediatrics at Sapporo Medical University Hospital or Department of Hematology and Oncology at the Children's Medical Center of Japanese Red Cross Nagoya First Hospital were retrospectively analyzed. Grading of GVHD was based on the report from the 1994 Consensus Conference.[5)]

Blood samples were obtained from patients undergoing allogeneic HSCT in the two hospitals after obtaining informed consent from the patients or their parents. The Ethics Committee of both hospitals approved the human sera studies. Sera had been collected on at least two occasions, aliquoted, and stored at －80ºC until use in assays. Human CCL8 enzyme-linked immunosorbent assay

(ELISA) kits were obtained from Immuno-Biological Laboratories (Gunma, Japan) and used according to the manufacturer's protocol.[6]

We retrospectively analyzed patient deaths that occurred within 180 days after HSCT. To examine any possible correlation between serum CCL8 level and the NRM of these patients, we chose the largest value of serum CCL8 acquired in the early phase of aGVHD as a representative CCL8 concentration for an individual case. Thus, serum CCL8 hereafter corresponds to the largest value of serum CCL8 amongst each patient's samples.

Statistical analysis was performed using JMP® 9 (SAS Institute, Cary, NC). The cut-off value of serum CCL8 correlating with NRM was determined by generation of a Receiver Operating Characteristic (ROC) curve. The Kaplan-Meier method was used to estimate survival rate, while log-rank and generalized Wilcoxon tests were used to assess differences in survival among patients groups. A landmark analysis method was used to assess the impact of CCL8 levels on survival by correcting for inherent bias in the analysis of time-to-event outcome between groups.[7] For landmark analysis, we set the landmark time to day 100 after HSCT because aGVHD is defined to develop within 100 days after HSCT. Values for significant difference were set at $p < 0.05$.

3. Results

Patient characteristics are shown in Table I. The median age was 8 years (range 1–21 years). Eleven patients died of infection, hemorrhage, TAM or other complications within 180 days after HSCT. The remaining 20 patients included six patients who died after day 180 with causes of death including pneumonia (n = 1), TAM (n = 1), secondary brain tumor (n = 1), and recurrence of initial disease (n = 3). Only one patient (UPN24) suffered a relapse within 180 days after HSCT. The performance of CCL8 for prediction of NRM was assessed by quantifying the area under the ROC curve (area under the curve is 0.741, Figure 1A). The cutoff value for serum CCL8 concentration was 213 pg/mL, corresponding to a specificity of 50% and sensitivity of 100%, and the likelihood ratio of a positive test was 2.0. According to serum CCL8 level, patients were divided into two groups: Group 1 (n = 21) included patients with a serum CCL8 concentration > 213 pg/mL, while Group 2 (n = 10) included patients with a serum CCL8 concentration < 213 pg/mL. Median day of aGVHD onset was 15 days after HSCT in Group 1 and 20 days in Group 2 ($p = 0.08$). Median serum CCL8 concentration was 512.9 pg/mL in Group 1 and 111.3 pg/mL in Group 2, and average CCL8 was 561.6 pg/mL in Group 1 and 119.8 pg/mL in Group 2 ($p < 0.01$).

Figure 1B depicts days of post-HSCT (x-axis) and highest value of serum CCL8 concentration (y-axis) up to 180 days post-HSCT. The vertical solid line indicates day 100 post-HSCT. The horizontal dotted line corresponds to 213 pg/mL of CCL8. All cases (100%) exhibited the highest serum CCL8 value before day 100 post-HSCT during the observation period. Open circles correspond to individuals who were still alive at day 180, while closed circles indicate individuals who died within 180 days post-HSCT. The deaths of the eleven individuals who died within 180 days post-HSCT were all non-relapse associated.

Figure 1C shows the survival rate at 180 days post-HSCT in all the patients with grade II–IV aGVHD (n = 31). Group 1 (solid line, serum CCL8 > 213 pg/mL) showed a significantly lower survival rate at 180 days after HSCT compared with Group 2 (dashed line, serum CCL8 < 213 pg/mL) ($P < 0.01$, log-rank and Wilcoxon tests). This result suggests that serum CCL8 concentration can predict NRM before day 180.

Figure 1D shows the results of landmark analysis. The landmark point was set at day 100 after HSCT because aGVHD is defined as developing within 100 days after HSCT.

4. Discussion

We previously reported that serum CCL8 concentration closely correlates with the survival of GVHD model mice, suggesting that chemotactic activity of this C-C chemokine may play a role in tissue injury associated with GVHD.[4] Our findings here indicate that the largest serum CCL8 value obtained during the early phase of grade II–IV aGVHD could predict NRM within 180 days after HSCT. Indeed, serum CCL8 values were relatively high in the early phase of aGVHD and slowly decreased with immunosuppressive treatment (data not shown). These results suggest that the

Table I. Patients' Characteristics

Patient No	age at SCT	Sex	Underlying Disease	HSCT Source	GVHD prophylaxis	aGVHD onset day[a]	aGVHD grade	MAX CCL8[b]	Non-relapse deaths	MAX CCL8 day[a]	day[a] of death
UPN01	6	F	AML (M2)	MSD-BMT	MTX	32	3	213.74	yes	84	107
UPN02	2	F	AML (M4)	UR-CBT	FK+ shortMTX	11	3	825.68	yes	12	118
UPN03	4	F	FHL	UR-BMT	CsA+ shortMTX	9	3	271.03	yes	76	82
UPN04	5	M	ALL	UR-BMT	CsA+ shortMTX	22	3	515.5	yes	14	49
UPN05	5	F	ALL	UR-BMT	CsA+ shortMTX	22	3	299.07	yes	12	55
UPN06	7	M	CML	UR-BMT	FK+ shortMTX	12	3	339.41	yes	20	38
UPN07	10	F	ALL	UR-BMT	CsA+ shortMTX	12	4	512.9	yes	12	58
UPN08	11	F	ALL	UR-BMT	CsA+ shortMTX	10	4	840.1	yes	10	114
UPN09	12	F	ALL	mis-UR-BMT	CsA+ shortMTX	10	4	818.56	yes	65	132
UPN10	14	M	T-lymphoma	UR-BMT	FK+ shortMTX	10	3	593.7	yes	11	64
UPN11	21	M	AA>MDS	UR-BMT	CsA+ shortMTX	13	3	750.2	yes	15	145
UPN12	1	M	infantile ALL	mis-UR-BMT	FK+ shortMTX	97	3	73.94	no	14	
UPN13	3	M	AA	UR-BMT	CsA+ shortMTX	22	2	442.37	no	12	
UPN14	5	M	Ph-ALL	MSD-BMT	MTX	19	3	174.51	no	18	562
UPN15	5	M	Fanconi Anemia	UR-CBT	CsA+MMF	10	3	115.3	no	10	251
UPN16	6	F	AA	UR-BMT	CsA+ shortMTX	26	3	682.41	no	15	
UPN17	7	M	ALL	UR-BMT	FK+ shortMTX	12	2	1118.53	no	17	
UPN18	7	M	ALL	MSD-BMT	MTX	27	2	205.6	no	61	2962
UPN19	7	M	ALL	UR-CBT	FK+ shortMTX	5	3	736.82	no	12	
UPN20	8	M	AA	UR-BMT	CsA+ shortMTX	17	3	240.85	no	16	
UPN21	8	M	AML (M2)	MSD-BMT	MTX	26	3	160.79	no	20	
UPN22	8	M	AA	mis-BMT (CD34+)	CsA+ shortMTX	73	3	107.3	no	97	
UPN23	10	F	Ph-ALL	MSD-BMT	MTX	18	2	441.8	no	14	468
UPN24	10	M	AA>MDS (RAEB-t)	UR-BMT	CsA+ shortMTX	37	3	846.28	no	47	317
UPN25	10	M	AML (M2)	UR-BMT	FK+ shortMTX	21	3	457.52	no	13	
UPN26	10	M	CML	UR-BMT	FK+ shortMTX	19	3	85.1	no	19	
UPN27	11	F	AA	UR-BMT	CsA+ shortMTX	16	2	421.36	no	20	
UPN28	11	M	ALL	UR-BMT	CsA+ shortMTX+ mPSL	28	2	20.4	no	28	
UPN29	13	M	Ph-ALL	UR-BMT	FK+ shortMTX	25	2	100.99	no	20	
UPN30	17	F	ALL	MSD-BMT	CsA+ shortMTX	20	2	154.04	no	13	
UPN31	20	F	AA	UR-BMT	CsA+ shortMTX	111	3	425.67	no	48	1239

a) the day of HSCT is day0. The blanks mean that the patient lives, b) MAX CCL8 corresponds to the maximum level of serum CCL8 after the HSCT.
Abbreviations in the Table I
aGVHD: acute graft-versus-host disease
HSCT: hematopoietic stem cell transplantation
BMT: bone marrow transplantation
UR-BMT: unrelated BMT
MSD-BMT: matched sibling donor BMT
UR-CBT: unrelated cord blood stem cell transplantation
ALL: acute lymphoblastic leukemia
AML: acute myeloblastic leukemia
AA: aplastic amemia
MDS: myelodysplastic syndrome
CML: chronic myelocytic leukemia
FHL: familial hemophagocytic lymphohistiocytosis

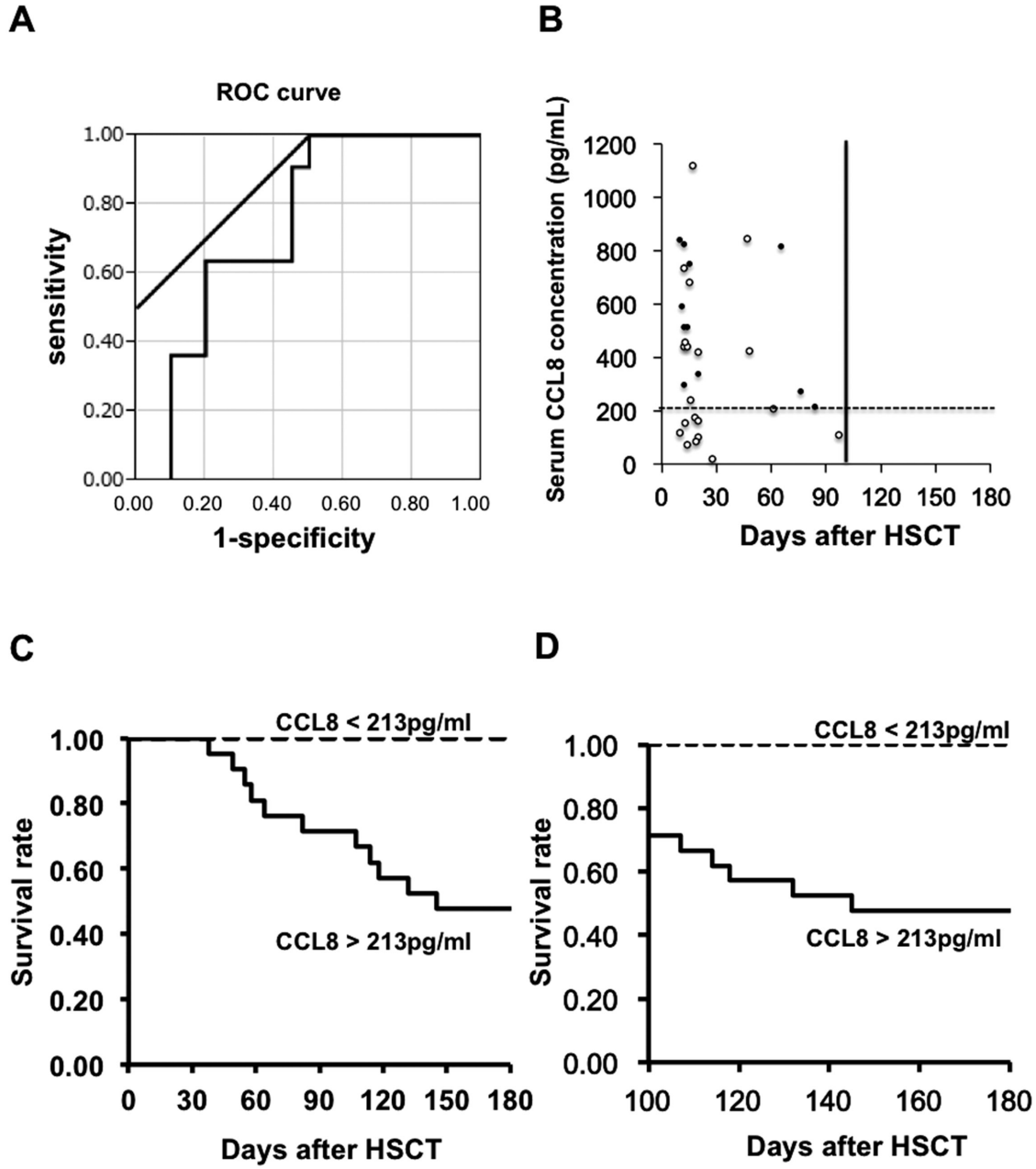

Figure 1.

A) Receiver operator characteristic (ROC) curve of Chemokine (C-C motif) ligand 8 (CCL8) versus non-relapse deaths areas. Area under the curve is 0.741. The cutoff value of serum CCL8 concentration is 213 pg/mL, corresponding to a specificity of 50% and sensitivity of 100%, and the likelihood ratio of a positive test is 2.0.

B) Distribution of maximum serum CCL8 concentration of each patient during the course of post-transplantation up to 180 days. Vertical solid line indicates day 100 post-transplantation. Horizontal dotted line indicates serum concentration of 213 pg/ml of CCL8. One circle corresponds to one patient. Open circles correspond to individuals alive at day 180, whereas closed circles indicate individuals who died within 180 days. All cases (100%) exhibited a maximum level of CCL8 before day 100 post-transplantation.

C) Evaluation of survival by Kaplan-Meier curve and log rank test. Patients with a maximum serum CCL8 concentration over 213 pg/mL (Group 1, solid line, CCL8 > 213 pg/ml, n = 21) showed a significantly lower survival rate at day 180 post-transplantation compared with individuals whose levels were lower than 213 pg /mL (Group 2, dashed line, CCL8 < 213 pg/ml, n = 10). Values of significance were similar in log rank (p = 0.0076) and Wilcoxon (p = 0.0087) tests.

D) Evaluation of the survival of 31 patients with grade II-IV aGVHD and recorded maximum serum CCL8 levels before day 100 post-transplantation by the landmark method at day 100. Patients with a maximum serum CCL8 concentration over 213 pg/mL (Group 1, solid line, CCL8 > 213 pg/ml, n = 21) showed a significantly lower survival rate at day 180 post-transplantation compared with individuals whose levels were less than 213 pg /mL (Group 2, dashed line, CCL8 < 213 pg/ml, n = 10). Values of significance were similar in log rank (p = 0.0076) and Wilcoxon (p = 0.0087) tests.

chemotactic activity and/or other functions of CCL8 could contribute quantitatively to aGVHD pathology, and are associated with prognosis. However, the precise mechanism by which CCL8 contributes to aGVHD has not been determined.

Glucksberg grading has been used to assess the clinical severity of aGVHD, with its maximum grade correlating with aGVHD prognosis.[8] However, the grade at onset is not helpful for prognostication. While several biomarkers for aGVHD have been proposed.[9-12], the absolute value of any single biomarker has not been found useful for evaluating aGVHD prognosis. Levine et al. reported a biomarker panel consisting of six biomarkers for predicting the treatment outcome and prognosis of aGVHD patients.[13] However, using a formula to assess the values of several biomarkers risks over-fitting. Nonetheless, there need to be more accurate studies regarding biomarkers and their potential usefulness for GVHD in clinical settings. In our results, the absolute value of CCL8 could predict the prognosis of aGVHD.

Although elevated serum CCL8 concentration within 100 days post-HSCT may predict NRM in patients with grade II–IV aGVHD, there are a few drawbacks of this cohort study. First, the sample size is too small to draw a definitive conclusion. Second, there might be a sampling bias, as this study was retrospective and the sampling times (days post-transplantation) varied. However, although there are some controversial points, our results are encouraging to launch a prospective observational study.

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Authorship Contributions

MY, and TH performed research.
MY, TH, NH, KI, NI, NS, KM and KK contributed vital new reagents.
MY, Y-M and YK performed statistical analysis.
MY, TH and YK designed research.
MY, TH and YK wrote the paper.

Conflicts of Interest Statement

All authors declare that they have no conflicts of interests.

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非血縁者間同種造血細胞移植後早期の血清中 CCL8 発現は移植後非再発死亡率と相関する

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急性移植片対宿主病（GVHD）におけるバイオマーカーとしてケモカイン CCL8 の役割を明らかにするため，grade II-IV の急性 GVHD 患者 31 名の血清と臨床経過を後方視的に解析した．血清 CCL8 濃度が，213pg/mL 以上であった 21 名中 11 名が移植後 180 日以内に死亡していたが，血清 CCL8 濃度が 213pg/mL 以下であった 10 名には死亡が見られなかった．ランドマーク解析でも CCL8 濃度が 213pg/mL 以上では明らかに生存率が低かった．移植後 100 日前の血清 CCL8 濃度上昇は急性 GVHD の予後を予見しうることが示唆された．