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# Diagnostic value of serum ferritin and the risk factors and cytokine profiles of hemophagocytic syndrome following allogeneic hematopoietic cell transplantation

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**Abstract**

To examine the diagnostic value of serum ferritin, the associated risk factors, and cytokine profiles of hemophagocytic syndrome (HPS) following allogeneic hematopoietic cell transplantation (allo-HCT), we retrospectively analyzed data from patients undergoing allo-HCT between 2006 and 2012. Of 223 eligible patients, 18 patients developed HPS. A serum ferritin level above 30,000 μg/l was highly specific for the detection of HPS (specificity, 93%). The one-year survival rate for HPS was significantly lower than that of non-HPS patients (37.5 % versus 72.9 %, respectively, Log-rank $P$<0.01). In multivariable Cox models, antigen mismatches in human leukocyte antigen in both graft-versus-host and host-versus-graft directions were significantly associated with the incidence of HPS. We found a significant elevation of Th1 cytokine (IFN-γ), Th2 cytokines (IL-10) and chemokines (MCP-1 and IP-10), at the onset of HPS. Our results suggest that allo-reactivity, derived from HLA-mismatch, and possibly causing a cytokine storm, may be associated with HPS development.

## INTRODUCTION

Secondary hemophagocytic syndrome (HPS), triggered by underlying diseases such as infections, malignancies, and rheumatic disorders [1, 2] occurs primarily in adults and is considered a potentially lethal cytokine-related disorder. The clinical hallmarks of secondary HPS include high fever, cytopenia, splenomegaly, and pathological findings of hemophagocytosis. Although the pathophysiology remains to be determined, failure to eliminate activated macrophages by natural killer (NK) cells and cytotoxic lymphocytes may lead to persistent abnormal activation of macrophages and excessive cytokine production by these cells [3–5].

Recently, several case reports and two retrospective studies have described that the emergence of HPS complicates allogeneic hematopoietic cell transplantation (allo-HCT); herein, the origin of the activated macrophages included both recipient-derived and donor-derived [6–9]. In addition, since post-allo-HCT HPS can lead to engraftment delay or failure, or multiple organ dysfunctions, the prognosis was reportedly poor [8, 9]. However, the contributing factors to post-allo-HCT HPS and the inflammatory process have not yet been adequately investigated. A diagnosis of HPS is generally made when a patient meets five or more criteria (of HLH-2004), consisting of eight non-specific criteria comprising fever, cytopenia, hypofibrinogenemia/hypertriglyceridemia, splenomegaly, hemophagocytosis, hyperferritinemia, elevated soluble interleukin-2 receptor α (sIL-2Rα), and decreased NK cell

activity [4]. Although these criteria have been internationally accepted as a highly useful diagnostic tool, the following disadvantages exist. Several of these criteria, especially hemophagocytosis, are absent at the onset of HPS in a large percentage of cases [10]; in addition, sIL-2Rα and NK function data may be unavailable in a timely manner in daily clinical practice [11]. Both of these obstacles could possibly lead to delayed therapeutic intervention and worsened prognosis. Moreover, in allo-HCT settings, before engraftment, it is difficult to accurately evaluate cytopenias or NK function, due to poor cell counts during conditioning chemotherapy. Takagi *et al*. used a criteria composed of two major (engraftment delay/failure and pathological findings of hemophagocytosis) and four minor criteria in accordance with several definitions including one by the FHL Study Group [12]. Although these modified criteria may not be suitable for earlier diagnosis, due to the requirement for information on engraftment delay/failure, it showed that most patients with HPS failed engraftment, resulting in poor prognosis after umbilical cord blood transplantation (CBT) [6]. An easier diagnostic tool for post-allo-HCT HPS and elucidation of more details on that are warranted. In addition, Allen *et al*. focused on the fact that serum ferritin levels may reflect stress-response and pro-inflammatory cytokines [13, 14], and showed excellent results in retrospective analysis; hyperferritinemia >10,000 mg/l was 90 % sensitive and 96 % specific for common HPS [15]. Therefore, a threshold serum ferritin level may be a useful marker for easy diagnosis of HPS, to initiate timely intervention. However, no study has examined the

optimal cut-off point of serum ferritin levels for diagnosing post-allo-HCT HPS.

Based on these observations, and using the modified HLH-2004 criteria for diagnosing post-allo-HCT HPS, we retrospectively investigated the diagnostic value of serum ferritin, risk factors, and the cytokine profiles of post-allo-HCT HPS.

## MATERIALS AND METHODS

### Study Design and Subjects

We retrospectively reviewed data from patients with hematological disorders who underwent allo-HCT at our institute between April 2006 and December 2012. We excluded patients who received double-unit umbilical CBT (n=3), rejected inclusion (n=2), or developed HPS before allo-HCT (n=5). The cohort consisted of 223 patients. Since it was difficult to obtain informed consent because it was a retrospective study, the plan and details of this study were announced to the public by displaying a notice at the hospital and on the website. This was in accordance with the ethical guidelines for epidemiological research compiled by 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.

**Transplantation Procedures**

A myeloablative regimen was defined as total body irradiation (TBI) 800 cGy in fractionated doses, oral or intravenous busulfan dose at > 9 mg/kg and > 7.2 mg/kg, respectively, a melphalan dose > 140 mg/m$^2$, or a thiotepa dose ≥ 10 mg/kg, according to the previous report [16]. For acute graft-versus-host disease (GVHD) prophylaxis, we mainly used calcineurin inhibitors (CIs) and methotrexate (MTX) or CIs and mycopheolate mofetil (MMF), and used posttransplantation cyclophosphamide only for recipients of haplo-identical donors, as described previously [17].

Donor sources included human leukocyte antigen (HLA)-matched or one-antigen mismatched related peripheral blood (rPB) / bone marrow (BM), haplo-identical rPB, unrelated BM, and umbilical CB. We defined a haplo-identical donor as a donor with first-degree relatives of a recipient (i.e. parent, child, or sibling), matched at 3/6 to 5/6 loci (HLA-A, -B or -DRB1) with a recipient, and was not an HLA 1-antigen mismatched sibling.

Disease risk was categorized into low-, intermediate- or high-risk according to the previous report [18]. Engraftment was defined as an absolute neutrophil count of at least 500/μL for three consecutive days. Primary engraftment failure was identified if a patient did not achieve successful engraftment, from transplantation to the last follow-up time or death. Delayed engraftment was defined as neutrophil engraftment on and after day 29. Acute GVHD was defined in agreement with standard criteria [19].

**Diagnosis of HPS**

As a rule, we diagnosed HPS according to the HLH-2004 criteria [4]. Briefly, HPS was diagnosed when a patient met at least five out of the following eight criteria: (1) fever, (2) splenomegaly, (3) cytopenias, (4) hypertrigleceridemia ≥265 mg/dl or hypofibrinogenemia ≤1.5 g/l, (5) histopathological findings of hemophagocytosis, (6) low or absent NK-cell activity, (7) hyperferritinemia ≥500 μg/l, and (8) high sIL-2R ≥2,400 U/ml. However, we could not apply these criteria to HPS development before engraftment, since it is difficult to perform an accurate evaluation on cytopenias or NK cell cytotoxicity, when patients are under the influence of pre-transplant conditioning or have poor cell counts in the peripheral blood. Moreover, serum levels of sIL-2R might be unreliable for diagnosing HPS in patients undergoing allo-HCT, because serum levels of sIL-2R are well known to be elevated in patients with acute GVHD [20]. Therefore, we identified pre-engraftment HPS using the following modified HLH-2004 criteria. We diagnosed HPS through histopathological findings of hemophagocytosis, and when a patient met at least three out of the following four criteria: (1) fever, (2) splenomegaly, (3) hypertrigleceridemia and/or hypofibrinogenemia, and (4) hyperferritinemia.

To assess underlying infection at the onset of HPS, we cultured samples obtained from the suspected sites of infection, and performed imaging tests and examined fungal markers

including plasma β-D glucan and serum *Aspergillus* galactomannan. Additionally, we performed polymerase chain reaction, testing for the presence of adenovirus, herpes simplex virus, varicella zoster virus, Epstein-Barr virus, human herpes virus 6, cytomegalovirus, and parvovirus B19 in the peripheral blood, and if available, also in samples from bone marrow.

**Monitoring of Serum Ferritin Levels and Measurements of Serum Cytokines and Chemokines**

We monitored serum ferritin levels using the architect ferritin assay system (Abbott Japan Co., Ltd. Chiba, Japan). Measurements were performed once a week during hospitalization and after allo-HCT, at day 30 ± 5 days, day 60 ± 7 days, day 90 ± 10 days, 180 ± 30 days, and thereafter every 12 months ± 2 months, or when an attending physician suspected the onset of HPS according to the institution's manual [21].

In available blood samples of patients with HPS, we comprehensively analyzed 20 serum cytokines and chemokines (Figure 3), determined by the Bio-Plex Pro Cytokine Assay® system (Bio-Rad Laboratories, CA); cut-off values for a positive identification were cited from the manufacturer's instruction [22]. Peripheral blood samples were collected before allo-HCT as baseline, and at onset of HPS. Samples at the onset were collected on the day of diagnosis ± 4 days. Blood samples were separated by centrifuging the blood at 3,000 rpm for

20 min, and samples were stored at -80 °C until analysis.

**Statistical analysis**

We used the Mann-Whitney U test for continuous variables or the Fisher's exact test for categorical variables, to examine the difference between patients who developed HPS and those who did not. To evaluate the impact of post-allo-HCT HPS on overall survival (OS), we performed a landmark analysis, which divided patients according to prior history of HPS, 30 days after transplant [23, 24]. This time was set in accordance with the definition of engraftment failure/delay, and the previous report [6]. The probability of OS was calculated by the Kaplan-Meier method and compared using the log-rank test. We used the Cox proportional hazards models with time-dependent covariates to investigate risk factors for HPS and to assess the impact of HPS on OS [23, 24]. Acute GVHD, use of ATG and HPS were treated as time-dependent covariates. The proportional hazards assumption was checked by Schoenfeld residuals. We assessed multicollinearity by inspecting the changes in the coefficient and standard error of each variable by the addition of another variable in each multivariable model, and by calculating the variance inflation factor [25, 26]. We used receiver operating characteristic (ROC) curves to assess whether serum ferritin levels could be used to accurately diagnose HPS, and calculated the optimal cutoff point by the Youden index. We employed Wilcoxon signed sum rank test to compare the differences in serum

levels of cytokines and chemokines between baseline values and those at onset of HPS.

All *P*-values and 95% confidence intervals were two-tailed. A value of $P<0.05$ was considered statistically significant. Statistical analyses were performed using Graph Pad Prism version 5.0 (Graph Pad Software, San Diego, CA, USA), and STATA SE version 13.0 (StataCorp, College Station, TX, USA).

## RESULTS

### Study Patients

A total of 223 patients were evaluable and 18 patients (8.1%) developed HPS. The characteristics of patients are shown in Table 1. Of the 18 patients with HPS, 14 patients had the following documented infections at the onset of HPS: bacterial in five, viral in six, fungal in two, and bacterial and viral in one. The median time from transplantation to HPS onset was 24.5 (range, 5–910) days. Nine patients developed HPS before engraftment. Of the nine patients, three failed and one delayed engraftment; all four patients underwent CBT.

The group of patients who developed HPS had a higher proportion of 2–3 antigen mismatches in both host-versus-graft (HVG) and GVH directions, and CIs plus MMF as GVHD prophylaxis, than those who did not. Of note, there were zero HLA-matched or -one antigen mismatched sibling PB or BM in the group of patients who developed HPS.

Furthermore, the group of patients who developed HPS had a higher proportion of two or more allele mismatches in both HVG and GVH directions, use of ATG before the onset of HPS, and had a lower incidence of acute GVHD before onset of HPS.

**Treatment and Outcome of HPS**

Of the 18 patients with HPS, 16 were treated with additional immunosuppressive therapy and the remaining two stayed on their original immunosuppressant therapy. Of the 16 patients, eight received steroids, including some at a high dose, and one was treated with steroids followed by second allo-HCT. The remaining seven patients received immunosuppressive therapy including etoposide. Four patients underwent etoposide alone, two were treated with etoposide and steroids, and one was treated with etoposide and ATG.

Median follow-up time was 302 days (range, 1–2,352 days) after transplantation. The prognosis in the HPS group was significantly worse than that of the non-HPS group (Fig 1) (1-year OS, 37.5 % versus 72.9 %, Log-rank $P<0.01$). In a multivariable Cox model, HPS was an independent prognostic factor for overall survival (Hazard ratio (HR) 4.49, 95 % CI, 2.30–8.76, $P<0.001$), adjusted for age (HR 1.02, 95 % CI, 1.00–1.05, $P=0.03$) and disease risk (high-risk (vs low-intermediate risk) HR 3.02, 95 % CI, 1.82–5.00, $P<0.001$). Finally, 13 patients died, and the cause of death was primary engraftment failure in three patients, infections in five, non-infectious pneumonia in two, relapse of primary disease in two, and

secondary cancer in one.

**Serum Ferritin Levels and Diagnosis of post-allo-HCT HPS**

Serum ferritin levels were monitored during follow up of allo-HCT in 220 patients; three patients who did not develop HPS were excluded because of missing information. Serum ferritin levels upon diagnosis of HPS were significantly higher than those of the non-HPS group after allo-HCT (Table I).

To examine the diagnostic performance of serum ferritin for allo-HCT HPS, we employed ROC analyses (Figure 2) and calculated the sensitivity and specificity at a certain cut-off value of ferritin (Table II). The area under the ROC curve of serum ferritin for HPS was 0.848 (95%CI, 0.725–0.971; $P<0.001$) (Figure 2), and the best cut-off value was calculated as 30,939 μg/l, which had a sensitivity of 72.2 % (95 % CI, 46.5–90.3) and a specificity of 93.6 % (95 % CI, 89.2–96.5) (Table II).

**Risk Factors for allo-HCT HPS**

In univariable Cox models, recipients of HLA 2–3 antigen mismatched donors in both GVH and HVG directions, an HLA 2 or more allele mismatched donor in the GVH direction, haplo-identical donor, and CB, and the use of ATG and MMF (versus MTX) were significantly associated with development of HPS (Table III). Of these significant variables,

there was significant multicollinearity between HLA disparity in the GVH direction, HVG direction, and donor sources. We therefore were unable to compare these variables in the same model. In addition, we were unable to assess accurately multivariable models, including HLA allele, due to missing information (approximately 12 %). In multivariable models 1a and 1b, both HLA 2–3 antigen mismatched donors in the GVH direction and the use of MMF were significantly associated with development of HPS (Table III). In models 2a and 2b, although the use of ATG was not significant, HLA 2–3 antigen mismatched donors in the HVG direction were significantly associated, and use of MMF was marginally, but significantly associated with development of HPS (Table III). In model 3, CB and use of MMF were marginally, but significantly associated with development of HPS (Table III).

**Serum cytokines and chemokines during follow-up of allo-HCT HPS**

A total of 16 samples from eight patients with HPS were available. Of the eight patients, six patients developed HPS before engraftment. In the eight patients who had samples both at baseline and at onset, the median levels of T helper cell 1 (Th1) cytokine (IFN-γ), Th2 cytokines (IL-5, IL-10), inflammatory cytokines (IL-1ra), and chemokines (IL-8, IP-10, MCP-1) were significantly elevated at disease onset when compared to baseline values. In addition, the elevation in the median levels of inflammatory cytokine (IL-6) was marginally significant at onset (Figure 3).

## DISCUSSION

In the present study, we demonstrated the following three points. First, serum hyperferritinemia with a higher cut-off level was highly specific for post-allo-HCT HPS. Second, HLA antigen mismatch in both directions was significantly associated with the incidence of post-allo-HCT HPS. Finally, in addition to both Th1- and Th2-cytokines, chemokines such as MCP-1, IP-10, or IL-8 were significantly elevated at the onset of post-allo-HCT HPS.

Compared with the previously reported serum ferritin cut-off level of 10,000 μg/l, which displayed high sensitivity and specificity for diagnosing HPS in children [15], the cut-off level of 30,000 μg/l for post-allo-HCT HPS determined in this study was much higher with similar specificity, but was less sensitive. This higher level may be explained partly by the fact that Asians may have higher serum ferritin levels than Caucasians [27]. In addition, it is thought that an uncontrolled, hyperimmune state, including elevated levels of multiple cytokines and chemokines, is initiated by Th1 cells, Th2 cells, macrophages, cytotoxic T cells and NK cells, in the pathophysiology of common HPS [3, 5, 28, 29]. The cytokine/chemokine data of patients with post-allo-HCT HPS in this study also showed elevations of broad-spectrum of cytokines and chemokines. An alloimmune reaction different

from GVHD might amplify abnormal immune activation, possibly leading to such hyperferritinemia. Paczesny *et al.* screened 120 plasma proteins at the diagnosis of acute GVHD and found that eight proteins including sIL-2R, TNFR1 and IL-8 were significantly elevated in patients with acute GVHD when compared to those of patients without GVHD [20]. Comparing this report with our data, MCP-1 and IP-10, possibly reflecting macrophage activation, might be candidate biomarkers to differentiate HPS from acute GVHD.

Notably, in our study subjects, no patients transplanted from HLA-matched or -one antigen mismatched sibling donors developed HPS. In addition, from multivariable Cox regression analyses, HLA antigen mismatch in both directions was identified as an independent risk factor, adjusted for GVHD as a time-dependent variable (data not shown). This result may explain the high incidence (cumulative incidence, 16.8 %) of HPS following CBT reported by Takagi *et al.*, as 87 % (103/119) of patients were transplanted with HLA-two antigen mismatch umbilical CB [6]. Moreover, the use of MMF as GVHD prophylaxis (vs. MTX) showed borderline significance in multivariable Cox models, most likely due to lack of adequate sample size. In addition, use of ATG was significantly associated with incidence of HPS in univariable analysis, whereas use of ATG was not significant in multivariable analysis, possibly due to lack of adequate sample size. To date, the exact mechanisms of post-allo-HCT HPS development remain unclear. Based on these observations, our data suggests that in addition to previous factors such as infections,

allo-reactivity derived from HLA-mismatch may contribute to the development of post-allo-HCT HPS. This is probably via activation of donor- and/or recipient-derived T cells and macrophages and hypercytokinemia. Furthermore, since the use of MMF as GVHD prophylaxis reportedly resulted in more severe GVHD than MTX [30], the weaker immunosuppressive effect of MMF might be associated with the development of post-allo-HCT HPS. In addition, T cell depletion caused by administration of ATG may also contribute to the development of HPS, although this remains unclear.

In the present study, the prognosis of patients with HPS was very poor, similar to previous reports [6, 9]. It is possible that optimal GVHD prophylaxis, with sufficient immunosuppressive effect in each individual transplant setting, might decrease the incidence of post-allo-HCT HPS, probably leading to improved prognosis for HPS. Further research is required to confirm this.

The nature of retrospective data and the definition of pre-engraftment HPS may limit the interpretation of our results. However, since our modified criteria required four out of the five selected HLH-2004 criteria (findings of hemophagocytosis were essential) to diagnose HPS, these modified criteria may be harder to meet than meeting five out of the eight criteria of the standard HLH-2004 criteria. Consequently, this study might have underdiagnosed true allo-HCT HPS cases. Validated diagnostic criteria for allo-HCT HPS are therefore needed for further investigations. Nevertheless, the present study was able to evaluate the impact of

HLA mismatch on the development of post-allo-HCT HPS since the study population included several combinations of recipient- and donor-HLA disparities.

Collectively, we have provided evidence that serum ferritin levels above 30,000 μg/l may be a specific diagnostic marker for HPS following allo-HCT. In addition, allo-reactivity derived from both host- and graft-directed HLA-mismatch, possibly causing a cytokine storm, may be associated with HPS incidence.

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## References

[1] Dhote R, Simon J, Papo T, et al. Reactive hemophagocytic syndrome in adult systemic disease: report of twenty-six cases and literature review. Arthritis Rheum 2003; 49: 633-639.

[2] Usmani GN, Woda BA, Newburger PE. Advances in understanding the pathogenesis of HLH. Br J Haematol 2013; 161: 609-622.

[3] Osugi Y, Hara J, Tagawa S, et al. Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. Blood 1997; 89: 4100-4103.

[4] Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr blood Cancer 2007; 48: 124-131.

[5] Tang Y, Xu X, Song H, et al. Early diagnostic and prognostic significance of a specific Th1/Th2 cytokine pattern in children with haemophagocytic syndrome. Br J Haematol 2008; 143: 84-91.

[6] Takagi S, Masuoka K, Uchida N, et al. High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. Br J Haematol 2009; 147: 543-553.

[7] Redjoul R, Toma A, Hicheri Y, et al. Hemophagocytic syndrome after allogeneic hematopoietic cell transplantation: more a graft rejection than an infectious process? Eur J Haematol 2012; 88: 458-460.

[8] Koh H, Nakane T, Sakamoto E, et al. Serum cytokine profiles in hemophagocytic syndrome following allogeneic hematopoietic stem cell transplantation. Acta Haematol 2012; 127: 182-185.

[9] Kobayashi R, Tanaka J, Hashino S, et al. Etoposide-containing conditioning regimen reduces the occurrence of hemophagocytic lymphohistiocytosis after SCT. Bone Marrow Transplant 2014; 49: 254-257.

[10] Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. Annu Rev Med 2012; 63: 233-246.

[11] Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis. Arch Dis Child 2011; 96: 688-693.

[12] Henter JI, Elinder G, Söder O, Hansson M, Andersson B, Andersson U. Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. Blood 1991; 78: 2918-2922.

[13] Tsuji Y, Ayaki H, Whitman SP, Morrow CS, Torti SV, Torti FM. Coordinate transcriptional and translational regulation of ferritin in response to oxidative stress. Mol Cell Biol 2000; 20: 5818-5827.

[14] Kwak EL, Larochelle DA, Beaumont C, Torti SV, Torti FM. Role for NF-kappa B in the regulation of ferritin H by tumor necrosis factor-alpha. J Biol Chem 1995; 270: 15285-15293.

[15] Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2008; 50: 1227-1235.

[16] Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transplant, 2009; 15: 367-369.

[17] Nakamae H, Koh H, Katayama T, et al. HLA haplo-identical peripheral blood stem cell transplantation using reduced dose of post-transplantation cyclophosphamide for poor prognosis or refractory leukemia and myelodysplastic syndrome. Exp Hematol 2015; 43: 921-929.

[18] Parimon T, Au DH, Martin PJ, Chien JW. A risk score for mortality after allogeneic hematopoietic cell transplantation. Ann Intern Med 2006; 144: 407-414.

[19] Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995; 15: 825-828.

[20] Paczesny S, Krijanovski OI, Braun TM, et al. A biomarker panel for acute graft-versus-host disease. Blood 2009; 113: 273-278.

[21] Nakamae M, Nakamae H, Koh S, et al. Prognostic value and clinical implication of serum ferritin levels following allogeneic hematopoietic cell transplantation. Acta Haematol 2015; 133: 310-316.

[22] Chapman P, Reyes C, Gupta V. Normal physiological levels of human cytokines using Bio-Plex Pro™ cytokine assays. Bio-Plex® suspension array system. Bio-Rad Tech Note 2010 - [cited 30 March 2016]; Available from: http://www.biorad.com/webroot/web/pdf/lsr/literature/Bulletin_6029.pdf.

[23] Storer BE. Statistical considerations in studies of late effects in HCT. Biol Blood Marrow Transplant 2009; 15 (1 Suppl): 25-28.

[24] Iacobelli S; EBMT Statistical Committee. Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 2013; 48 Suppl 1: S1-37.

[25] Armitage P, Berry G, Matthews JNS. Statistical Methods in Medical Research, Fourth Edition. Blackwell Publications: Oxford; 2002. p 358–360.

[26] O'brien RM. A caution regarding rules of thumb for variance inflation factors. Quality & Quantity 2007; 41: 673-690.

[27] Harris EL, McLaren CE, Reboussin DM,et al. Serum ferritin and transferrin saturation in Asians and Pacific Islanders. Arch Intern Med 2007; 167: 722-726.

[28] Tamura K, Kanazawa T, Tsukada S, Kobayashi T, Kawamura M, Morikawa A. Increased serum monocyte chemoattractant protein-1, macrophage inflammatory protein-1beta, and interleukin-8 concentrations in hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2008; 51: 662-668.

[29] Takada H, Takahata Y, Nomura A, Ohga S, Mizuno Y, Hara T. Increased serum levels of interferon-gamma-inducible protein 10 and monokine induced by gamma interferon in patients with haemophagocytic lymphohistiocytosis. Clin Exp Immunol 2003; 133: 448-453.

[30] Perkins J, Field T, Kim J, Kharfan-Dabaja MA, et al. A randomized phase II trial comparing tacrolimus and mycophenolate mofetil to tacrolimus and methotrexate for acute graft-versus-host disease prophylaxis. Biol Blood Marrow Transplant 2010; 16: 937-947.

**Figure Legends**

**Figure 1. One-year overall survival curves in patients with and without a prior history of hemophagocytic syndrome (HPS) (within 30 days of allogeneic hematopoietic cell transplantation).**

**Figure 2. Receiver operating characteristic (ROC) analysis of serum ferritin.**

The area under the ROC curve of serum ferritin for HPS (solid line) was 0.848 (95%CI, 0.725–0.971; *P*<0.001). The dashed line represents the reference line.

**Figure 3. Serum levels of 20 cytokines and chemokines in eight cases during follow-up of hemophagocytic syndrome following allogeneic hematopoietic cell transplantation.**

The median levels of inflammatory cytokines at baseline were as follows [normal reference range*, pg/ml]: (A) Tumor necrosis factor (TNF)-α, 3.27 pg/ml (range, 0.00–33.26) [≤98.00]; (B) Interleukin (IL)-1 receptor antagonist (RA), 27.84 (0.00–61.13) [≤665.00]; (C) IL-1β, 0.42 (0.00–1.40) [<0.70]; and (D) IL-6, 2.95 (0.00–69.92) [≤9.00]. T helper (Th)1-related cytokines: (E) IL-2, 0.00 (0.00–16.27) [≤90.00]; (F) IL-12, 4.63 (0.00–17.74) [≤6.00]; and (G) Interferon (IFN)-γ, 15.94 (3.28–61.77) [≤124.00]. Th2-related cytokines: (H) IL-4, 0.71

(0.19–5.09) [≤3.00]; (I) IL-5, 1.24 (0.00–7.39) [≤7.00]; (J) IL-10, 1.73 (0.00–4.57) [≤2.00]; and (K) IL-13, 1.74 (0.00–14.43) [≤9.00]. Th17-related cytokines: (L) IL-17, 0.00 (0.00–163.05) [≤31.00]. Other cytokines: (M) IL-7, 4.34 (1.97–11.69) [≤13.00]; (N) IL-9, 0.93 (0.00–4.40) [≤500.00]; and (O) IL-15, 0.00 (0.00–60.49) [≤5.00]. Chemokines: (P) IL-8, 6.98 (1.64–121.39) [≤116.00]; (Q) IFN-γ inducible protein (IP)-10, 780.00 (419.81–2,845.65) [≤637.00]; (R) Monocyte chemoattractant (MCP)-1, 42.70 (12.77–152.02) [≤48.00]; (S) Macrophage inflammatory protein (MIP)-1α, 2.26 (0.00–10.77) [<2.00]; and (T) MIP-1β, 102.31 (32.08–241.20) [≤47.00].

The median levels of inflammatory cytokines at onset were as follows: (A) TNF-α, 16.95 pg/ml (range, 0.56–160.34); (B) IL-1RA, 77.74 (6.90–310.46); (C) IL-1β, 0.59 (0.10–3.46); and (D) IL-6, 49.32 (10.77–174.94). Th1-related cytokines: (E) IL-2, 0.00 (0.00–2.95); (F) IL-12, 3.80 (0.00–17.75); and (G) IFN-γ, 40.57 (2.92–117.11). Th2-related cytokines: (H) IL-4, 1.54 (0.00–4.16); (I) IL-5, 36.32 (1.61–146.05); (J) IL-10, 9.03 (3.50–81.17); and (K) IL-13, 2.17 (0.81–7.69). Th17-related cytokines: (L) IL-17, 0.00 (0.00–2.54). Other cytokines: (M) IL-7, 7.06 (2.35–24.82); (N) IL-9, 4.68 (0.09–81.60); and (O) IL-15, 23.48 (0.00–75.70). Chemokines: (P) IL-8, 63.68 (21.38–437.31); (Q) IP-10, 6,721.01 (1,311.68–27,831.27); (R) MCP-1, 166.67 (28.41–826.52); (S) MIP-1α, 4.71 (0.73–21.83); and (T) MIP-1β, 167.26 (62.83–267.82).

*The upper limit data are cited from the serum data of healthy controls (reference 22).

**Table I. Baseline characteristics of study patients according to hemophagocytic syndrome (HPS) status during the follow-up period**

| Characteristic | HPS (-) | HPS (+) | P values |
|---|---|---|---|
| Number of patients, n | 205 | 18 | |
| Male, n (%) | 109 (53.2) | 12 (66.7) | 0.329 |
| Median age at transplantation (range), | 45 (16–69) | 49.5 (22–64) | 0.359 |
| years Primary diseases, % | | | 0.760 |
| Acute myelogenous leukemia | 89 (43.4) | 7 (38.9) | |
| Myelodysplastic syndrome | 23 (11.2) | 3 (16.7) | |
| Acute lymphoblastic leukemia | 32 (15.6) | 2 (11.1) | |
| Malignant lymphoma | 38 (18.5) | 5 (27.8) | |
| Others* | 23 (11.2) | 1 (5.6) | |
| Disease Status, n (%) | | | 0.754 |
| Low | 19 (9.3) | 1 (5.6) | |
| Intermediate | 115 (56.1) | 9 (50.0) | |
| High | 71 (34.6) | 8 (44.4) | |
| Blood type, n (%) | | | 0.607 |
| Match | 123 (60.0) | 11 (61.1) | |
| Major mismatch | 29 (14.2) | 1 (5.6) | |
| Minor mismatch | 34 (16.6) | 5 (27.8) | |
| Major-minor mismatch | 19 (9.3) | 1 (5.6) | |
| Donor, n (%) | | | 0.017 |
| HLA-matched or -one antigen mismatched sibling PB or BM | 46 (22.4) | 0 (0.0) | |
| Unrelated BM | 93 (45.4) | 7 (38.9) | |

| | | | |
|---|---|---|---|
| Umbilical cord blood | 46 (22.4) | 7 (38.9) | |
| Haplo-identical PB | 20 (9.8) | 4 (22.2) | |
| HLA mismatch at antigen level | | | |
| GVH direction (HLA-A, -B and -DR antigen), n (%) | | | 0.011 |
| 0–1 antigen mismatch | 153 (74.6) | 8 (44.4) | |
| 2–3 antigen mismatch | 52 (25.4) | 10 (55.6) | |
| HVG direction (HLA-A, -B and -DR antigen), n (%) | | | 0.006 |
| 0–1 antigen mismatch | 149 (72.7) | 7 (38.9) | |
| 2–3 antigen mismatch | 56 (27.3) | 11 (61.1) | |
| HLA mismatch at allele level | | | |
| GVH direction (HLA-A, -B and -DR allele), n (%) | | | 0.109 |
| 0–1 allele mismatch | 123 (60.0) | 7 (38.9) | |
| 2 or more allele mismatch | 56 (27.3) | 9 (50.0) | |
| Missing | 26 (12.7) | 2 (11.1) | |
| HVG direction (HLA-A, -B and -DR allele), n (%) | | | 0.329 |
| 0–1 allele mismatch | 122 (59.5) | 8 (44.4) | |
| 2 or more allele mismatch | 57 (27.8) | 8 (44.4) | |
| Missing | 26 (12.7) | 2 (11.1) | |
| Conditioning regimen, n (%) | | | 0.748 |
| Myeloablative | | | |
| Total-body irradiation based | 57 (27.8) | 4 (22.2) | |
| Busulfan-based | 104 (50.7) | 9 (50.0) | |
| Melphalan- or thiotepa-based | 3 (1.5) | 0 (0.0) | |

| | | | |
|---|---|---|---|
| Reduced-intensity | | | |
| Fludarabine based | 34 (16.6) | 5 (27.8) | |
| Others | 7 (3.4) | 0 (0.0) | |
| GVHD prophylaxis, n (%) | | | 0.023 |
| Calcineurin inhibitor with MTX | 142 (69.3) | 7 (38.9) | |
| Calcineurin inhibitor with MMF | 28 (13.7) | 5 (27.8) | |
| Others† | 35 (17.1) | 6 (33.3) | |
| Use of ATG before diagnosis of HPS, n (%) | 37 (18.1) | 7 (38.9) | 0.057 |
| Administration of ATG prior to transplantation | 31 (15.1) | 5 (27.8) | 0.180 |
| ATG for GVHD treatment | 6 (2.9) | 2 (11.1) | 0.129 |
| Acute GVHD prior to diagnosis of HPS, n (%) | 150 (73.2) | 10 (55.6) | 0.169 |
| Serum ferritin level at diagnosis of HPS‡, μg/l (range) | 7,664 (329–126,035) | 40,166 (2,530–184,186) | <0.001 |

HPS, hemophagocytic syndrome; HLA, human leukocyte antigen; GVH, graft-versus-host; HVG, host-versus-graft; PB, peripheral blood; BM, bone marrow; GVHD, graft-versus-host disease; MTX, methotrexate; MMF, mycophenolate mofetil; ATG, antithymocyte globulin.

*Others included chronic myelogenous leukemia, aplastic anemia, chronic active Epstein-Barre virus infection, myelofibrosis, chronic neutrophilic leukemia.

†Others included calcineurin inhibitor alone and posttransplantation cyclophosphamide.

‡Serum ferritin levels at the diagnosis of HPS and at the maximum after HCT were used in the HPS group (n=18) and in the non-HPS group (n=220 (missing information, n=3)), respectively.

**Table II. Diagnostic performance of serum ferritin levels for diagnosis of hemophagocytic syndrome at onset according to different cut-off points (n=220)**

| Ferritin cut off value, µg/l | Sensitivity, % (95% CI) | Specificity, % (95% CI) | Positive predictive value, % (95% CI) | Negative predictive value, % (95% CI) |
|---|---|---|---|---|
| 10,000 | 83.3 (58.6–96.4) | 60.4 (53.3–67.2) | 15.8 (9.1–24.7) | 97.6 (93.1–99.5) |
| 20,000 | 77.8 (52.4–93.6) | 85.1 (79.5–89.8) | 31.8 (18.6–47.6) | 97.7 (94.3–99.4) |
| 30,000 | 72.2 (46.5–90.3) | 93.1 (88.6–96.2) | 48.1 (28.7–68.1) | 97.4 (94.1–99.2) |
| 40,000 | 50.0 (26.0–74.0) | 96.0 (92.3–98.3) | 52.9 (27.8–77.0) | 95.6 (91.8–98.0) |
| 50,000 | 44.4 (21.5–69.2) | 96.5 (93.0–98.6) | 53.3 (26.6–78.7) | 95.1 (91.2–97.6) |
| Best cut off value 30,939 Youden index 0.658 | 72.2 (46.5–90.3) | 93.6 (89.2–96.5) | 50.0 (29.9–70.1) | 97.4 (94.1–99.2) |

CI, confidence interval.

**Table III. Univariable and multivariable Cox models of hemophagocytic syndrome incidence following allogeneic hematopoietic cell transplantation (n=223)**

| Factors | Hazard Ratio (95% CI) | *P* value |
|---|---|---|
| **Univariable analysis** | | |
| Age at transplantation | 1.02 (0.98–1.05) | 0.396 |
| Gender | | |
| Male | 1.00 (reference) | |
| Female | 0.56 (0.21–1.51) | 0.253 |
| Analysis at antigen level (HLA-A, -B and -DR antigen) | | |
| GVH direction (HLA-A, -B and -DR antigen) | | |
| 0–1 antigen mismatch | 1.00 (reference) | |
| 2–3 antigen mismatch | 3.98 (1.55–10.20) | 0.004 |
| HVG direction (HLA-A, -B and -DR antigen) | | |
| 0–1 antigen mismatch | 1.00 (reference) | |
| 2–3 antigen mismatch | 4.53 (1.74–11.81) | 0.002 |
| Analysis at allele level (HLA-A, -B and -DR allele)* | | |
| GVH direction (HLA-A, -B and -DR allele) | | |
| 0–1 allele mismatch | 1.00 (reference) | |
| 2 or more allele mismatch | 3.01 (1.12–8.13) | 0.029 |
| HVG direction (HLA-A, -B and -DR allele) | | |
| 0–1 allele mismatch | 1.00 (reference) | |
| 2 or more allele mismatch | 2.28 (0.85–6.10) | 0.100 |
| Donor | | |

| | | |
|---|---|---|
| HLA-matched or one antigen mismatched sibling PB/BM and unrelated BM | 1.00 (reference) | |
| Umbilical cord blood | 3.26 (1.14–9.35) | 0.028 |
| Haplo-identical PB | 4.42 (1.27–15.39) | 0.019 |
| GVHD prophylaxis[†] | | |
| Calcineurin inhibitor with MTX | 1.00 (reference) | |
| Calcineurin inhibitor with MMF | 3.87 (1.20–12.44) | 0.023 |
| Use of ATG prior to diagnosis of HPS | | |
| No | 1.00 (reference) | |
| Yes | 3.36 (1.30–8.72) | 0.013 |
| Acute GVHD prior to diagnosis of HPS | | |
| No | 1.00 (reference) | |
| Yes | 1.96 (0.61–6.30) | 0.257 |
| **Multivariable analysis** | | |
| Model 1a | | |
| GVH direction (HLA-A, -B and -DR antigen) | | |
| 0–1 antigen mismatch | 1.00 (reference) | |
| 2–3 antigen mismatch | 3.19 (1.18–8.62) | 0.022 |
| Use of ATG prior to diagnosis of HPS | | |
| No | 1.00 (reference) | |
| Yes | 2.32 (0.85–6.33) | 0.102 |
| Model 1b | | |
| GVH direction (HLA-A, -B and -DR antigen) | | |

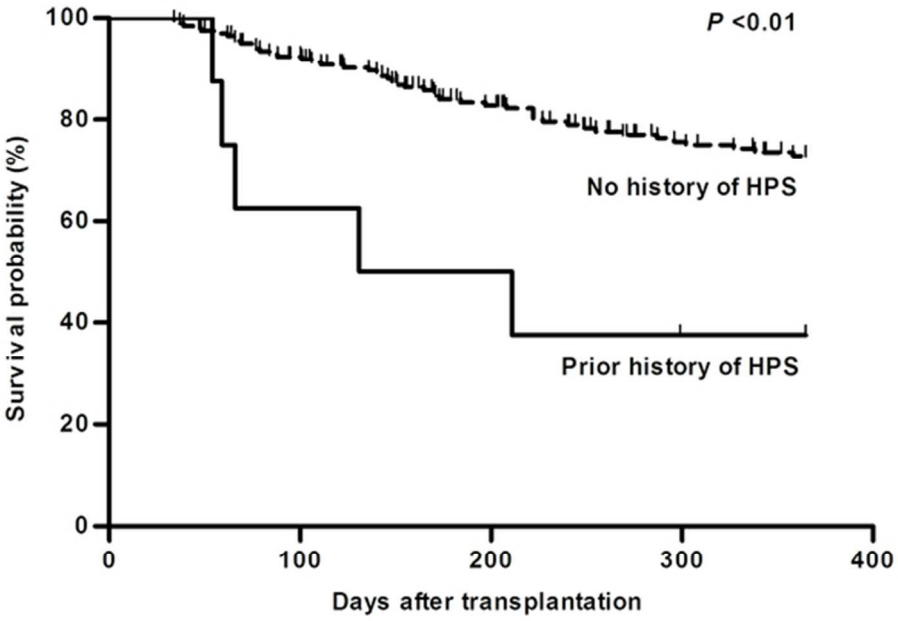
| | | |
|---|---|---|
| 0–1 antigen mismatch | 1.00 (reference) | |
| 2–3 antigen mismatch | 3.05 (1.11–8.37) | 0.030 |
| GVHD prophylaxis[†] | | |
| Calcineurin inhibitor with MTX | 1.00 (reference) | |
| Calcineurin inhibitor with MMF | 3.34 (1.02–10.92) | 0.046 |
| Model 2a | | |
| HVG direction (HLA-A, -B and -DR antigen) | | |
| 0–1 antigen mismatch | 1.00 (reference) | |
| 2–3 antigen mismatch | 3.65 (1.31–10.14) | 0.013 |
| Use of ATG prior to diagnosis of HPS | | |
| No | 1.00 (reference) | |
| Yes | 2.08 (0.75–5.76) | 0.158 |
| Model 2b | | |
| HVG direction (HLA-A, -B and -DR antigen) | | |
| 0–1 antigen mismatch | 1.00 (reference) | |
| 2–3 antigen mismatch | 3.39 (1.19–9.66) | 0.023 |
| GVHD prophylaxis[†] | | |
| Calcineurin inhibitor with MTX | 1.00 (reference) | |
| Calcineurin inhibitor with MMF | 2.99 (0.90–9.91) | 0.073 |
| Model 3 | | |
| Donor | | |
| HLA-matched or one antigen mismatched sibling PB/BM and unrelated BM | 1.00 (reference) | |
| Umbilical cord blood | 2.63 (0.89–7.77) | 0.079 |

| | | |
|---|---|---|
| Haplo-identical PB | 2.45 (0.43–13.87) | 0.312 |
| GVHD prophylaxis† | | |
| Calcineurin inhibitor with MTX | 1.00 (reference) | |
| Calcineurin inhibitor with MMF | 3.21 (0.98–10.53) | 0.055 |

HPS, hemophagocytic syndrome; HLA, human leukocyte antigen; GVH, graft-versus-host; HVG, host-versus-graft; PB, peripheral blood; BM, bone marrow; GVHD, graft-versus-host disease; MTX, methotrexate; MMF, mycophenolate mofetil; ATG, antithymocyte globulin.

* The analytic cohort included all subjects and the results were adjusted for the category of missing information (n=28; on HLA allele in the GVH and HVG direction).

† The analytic cohort included all subjects and the results were adjusted for the category of other GVHD prophylaxis (n=41; including calcineurin inhibitor alone or posttransplantation cyclophosphamide).

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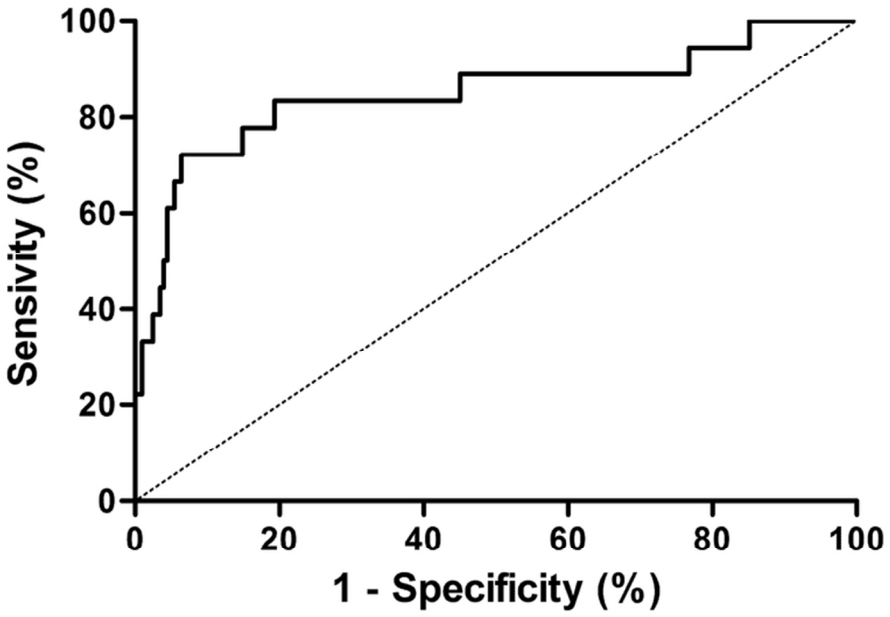


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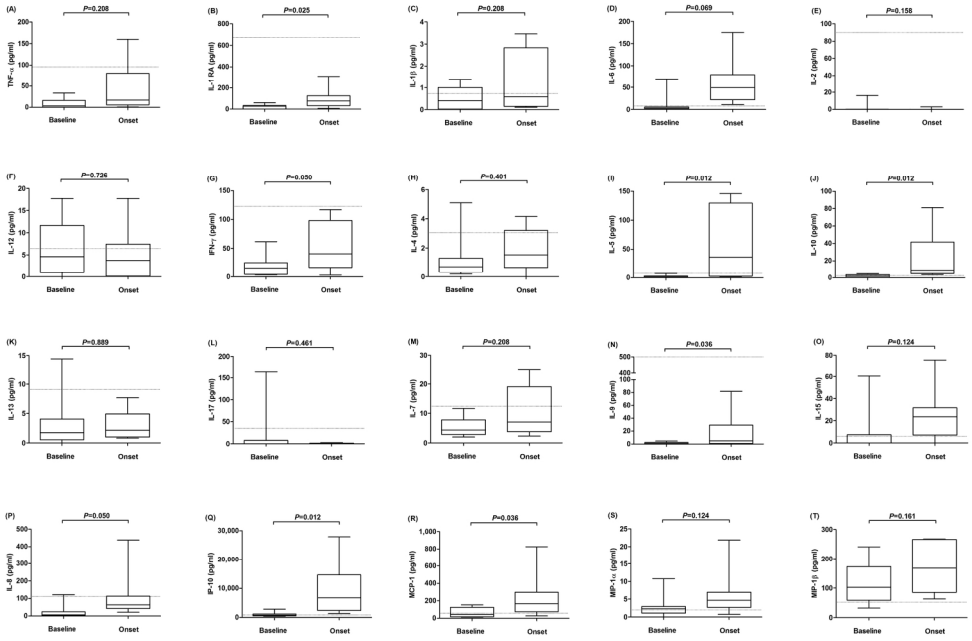


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