**Table 3. Outcomes after the Second SCT (RICBT)**

| Parameters | n = 80 |
|---|---|
| The engraftment rate in 61 patients surviving >28 days | 45 (74%) |
| GF in 61 patients surviving >28 days | 13 (21%) |
| Grade 3-4 organ toxicities* | 48 (60%) |
| Documented infection | 58 (63%) |
| CMV antigenemia | 36 (45%) |
| Acute GVHD | |
| Grade II-IV | 20 (25%) |
| Grade III-IV | 9 (11%) |
| Relapse | 12 (15%) |
| Death | 51 (64%) |
| The median day of death after second SCT | 37 days (range: 2-611) |
| Causes of death | |
| Infection | 33 (65%) |
| Bacterial | 14 |
| Fungal | 6 |
| Viral | 8 |
| Complex or unknown | 5 |
| Relapse | 6 (12%) |
| Acute GVHD | 1 (2%) |
| Other† | 11 (22%) |

SCT indicates stem cell transplantation; RICBT, reduced-intensity cord blood transplantation; GF, graft failure; CMV, cytomegalovirus; GVHD, graft-versus-host disease.
*Grade of organ toxicities was evaluated by the CTCAE v3.0 [40].
†Other causes included cerebral hemorrhage (n = 3), multiorgan failure (n = 2), thrombotic microangiopathy (n = 2), veno-occulusive disease of the liver (n = 1), interstitial pneumonitis (n = 1), heart failure (n = 1), and secondary malignancy (n = 1).

and only 1 patient who had grade IV aGVHD died of GVHD.

### TRM, Relapse, and Causes of Death (Table 3)

Fifty-one patients (64%) died at a median of 37 days (range: 2-611) after the second SCT. The cumulative incidence of TRM was 45%, 56%, and 61% at day 100, 1 year, and 2 years, respectively (Figure 2A), and infection was the most frequent cause of death. Notably, death that was directly related to bacterial infection occurred during prolonged neutropenia in the first 2 months after the second SCT. In 11 patients with grade 3 or 4 carryover organ toxicities at the second SCT, 8 (73%) died of TRM (Figure 2B). TRM was higher in patients who received an oral busulfan-based regimen (72%) than in those who received melphalan-based (50%) or cyclophosphamide-based (53%) regimens. Underlying malignancy relapsed in 12 patients (16%) at a median of 158 days (range: 22-781) after the second SCT, and 3 patients received a third SCT after relapse. Overall, 6 patients died of disease recurrence.

### Survival

The median follow-up time in the surviving patients was 325 days (range: 89-1069) after the second SCT. The Kaplan-Meier curves of OS and PFS of all 80 patients are shown in Figure 3A. The estimated

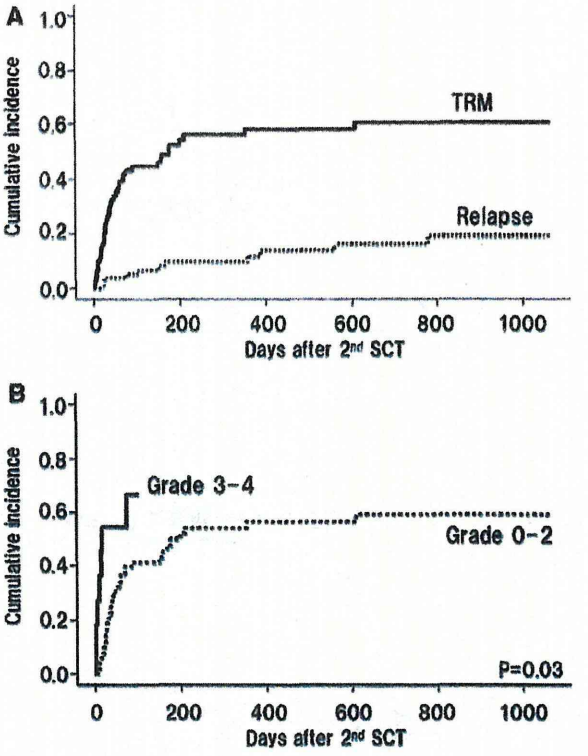


**Figure 2.** Cumulative incidence of transplantation-related mortality (TRM) and relapse. (A) The cumulative incidences of TRM (solid line) and relapse (dotted line) are shown. (B) The cumulative incidence of TRM was higher in patients who had grade 3 or 4 carryover organ toxicity before the second SCT (solid line) than in those who did not (dotted line) ($P = .03$).

rates of OS and PFS at 1 year after the second SCT were 33% and 29%, respectively. The OS was worse in 11 patients who had grade 3 or 4 carryover organ toxicities at the second SCT compared to the other 69 patients. OS was significantly better in patients who had standard-risk disease at the first SCT than in those who had high-risk disease (Figure 3B).

### Factors Associated with Engraftment and OS

In a univariate analysis, standard risk at the first SCT, PS 0-1 at the second SCT, conditioning that included alkylating agents or 2-4 Gy TBI, and a higher dose of infused TNC ($\geq 2.5 \times 10^7$/kg) were significantly associated with a higher probability of engraftment. Carryover organ toxicities ($P = .09$) and infection at the second SCT ($P = .07$) were also included in a multivariate analysis. The type of engraftment failure after first SCT did not have an influence on outcome after the second SCT (primary versus secondary). As a result, higher TNC dose ($\geq 2.5 \times 10^7$/kg: hazard ratio [HR] = 2.14, 95% confidence interval [CI], 1.29-3.52; $P = .003$), conditioning that included alkylating agents (HR = 3.70, 95% CI, 1.51-9.09; $P = .005$), and standard risk at first SCT (HR = 2.04, 95% CI, 1.06-3.85; $P = .03$)

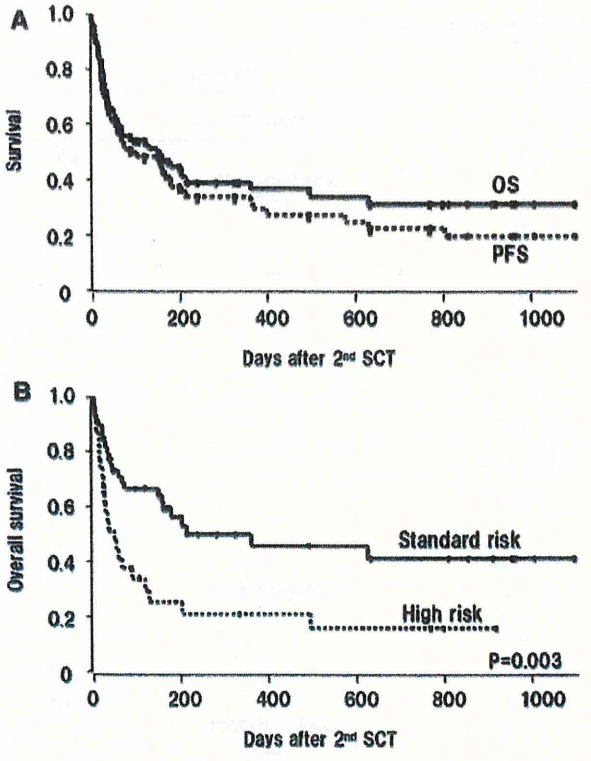


**Figure 3.** OS and PFS. (A) The Kaplan-Meier estimates of OS (solid line) and PFS (dotted line) are shown. (B) OS in patients who were high risk at the first SCT (dotted line) was lower than that in those who were standard risk (solid line) ($P = .003$).

remained significant in the multivariate Cox proportional hazards regression analysis (Table 4). In a multivariate Cox proportional hazards regression analysis of OS, high-risk disease at the first SCT (HR = 2.14, 95% CI, 1.20-3.81; $P = .01$) and grade 3 or 4 carryover organ toxicities at the second SCT (HR = 2.84, 95% CI, 1.33-6.06; $P = .007$) were associated with an increased risk of poor OS (Table 5).

## DISCUSSION

Based on data obtained from this large cohort of patients, we showed that neutrophil engraftment can be achieved in >70% of adult patients who received RICBT as salvage therapy for GF. Although our cohort was composed of rather older patients, the engraftment rate was comparable to that reported in primary CBT [17,18,29,34]. Considering the poor PS and carryover infection and organ toxicities, salvage therapy with RICBT is a feasible option that gave a 1-year OS of 33%. Nevertheless, this procedure is still associated with a high rate of TRM (45% at day 100), 60% of which was related to infectious complications, and we performed analyses to identify the risk factors for engraftment and survival.

Guardiola et al. [22] reported in 82 patients with various hematological diseases who underwent second allogeneic SCT that the neutrophil engraftment rate and 3-year OS were 70% and 30%, respectively. They showed that a longer intertransplant interval of ≥80 days was associated with a higher neutrophil engraftment rate and survival in a multivariate analysis. McCann et al. [19] also reported that a longer interval of ≥60 days was associated with a higher engraftment rate and OS in 41 patients with aplastic anemia. In our study, we did not find any association between interval and neutrophil engraftment or OS, and this discrepancy may be because of differences in the cohorts of patients evaluated. In the report by Guardiola et al. [22], the proportions of patients who experienced secondary GF and who received transplant from an HLA-matched sibling donor were much higher than in our study (66% versus 20%, 78% versus 6%, respectively). Grandage et al. [25] reported successful engraftment in 12 patients who underwent a second SCT from the same unrelated donor after GF. In the current study, however, it was not possible to perform a second SCT using an unrelated BM donor because most patients had poor PS, organ toxicities, or infections with prolonged cytopenia (ANC <100/mm$^3$).

Our data confirmed that a higher number of infused CB cells (TNC ≥2.5 × 10$^7$/kg) was associated with a higher probability of neutrophil engraftment after the second RICBT ($P = .01$), which was consistent with previous reports [4,42]. Because the median body weight of patients in this study was 55 kg, CB units containing >2.0 × 10$^7$/kg were available in >80% of patients. A double cord blood unit strategy might be favorable as previous reported, because a higher cell dose was associated with better survival [43]. Although in a previous study by Wagner et al. [44], the total number of CD34$^+$ cells was reported to be a major determinant of neutrophil recovery after CBT, our present findings did not confirm this point. Another discrepancy with previous reports [44] is that HLA disparity between the donor and recipient was not related to the engraftment rate in our study. We also examined the effect of HLA mismatch with serological HLA-A, B and allele DRB1 except for 5 patients whose allele typing was not performed. However, the results remained unchanged, and there was no impact on engraftment and OS.

The need for an intensive immunosuppressive conditioning regimen before the second SCT for GF depends on the mechanism of GF, and we found that a fludarabine-based regimen that included alkylating agents was associated with a higher neutrophil engraftment rate. Whereas the use of cytotoxic drugs is not mandatory before stem cell boost for patients who have poor graft function [23,24], intensive immunosuppressive conditioning is essential to suppress residual host T and natural killer cells to

**Table 4. Univariate and Multivariate Analysis of Factors Predicting Engraftment after the Second SCT**

| Covariates | Proportion (%)* | Univariate | Multivariate | |
|---|---|---|---|---|
| | | P | Hazard Ratio (95% CI) | P |
| Disease risk at the first SCT† | | .02 | | .03 |
| Standard risk | 70 | | 2.04 (1.06-3.85) | |
| High risk | 43 | | 1.00 | |
| Type of graft failure | | .57 | | — |
| Primary | 58 | | — | |
| Secondary | 56 | | — | |
| Interval between the first SCT and second SCT | | .87 | | — |
| <50 days | 60 | | — | |
| ≥50 days | 59 | | — | |
| PS | | .01 | | — |
| 0-1 | 81 | | — | |
| 2-4 | 46 | | — | |
| Carryover organ toxicities at the second SCT‡ | | .09 | | — |
| Grade 0-2 | 65 | | — | |
| Grade 3-4 | 27 | | — | |
| Carryover infection at the second SCT | | .07 | | — |
| Febrile neutropenia/none | 69 | | — | |
| Documented infection | 51 | | — | |
| Conditioning§ | | .0001 | | .005 |
| Alkylating agent–containing | 73 | | 3.70 (1.51-9.09) | |
| Other | 26 | | 1.00 | |
| TBI | | .03 | | — |
| 2-4 Gy TBI | 71 | | — | |
| No TBI | 50 | | — | |
| TNC of the CB | | .01 | | .003 |
| $\geq 2.5 \times 10^7$/kg | 73 | | 2.14 (1.29-3.52) | |
| $<2.5 \times 10^7$/kg | 50 | | 1.00 | |

SCT indicates stem cell transplantation; PS, performance status; TBI, total-body irradiation; TNC, total nucleated cells; CB, cord blood; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; CML, chronic myelogeneous leukemia; MDS, myelodysplatic syndrome; RAEB, refractory anemia with excess blasts; CI, confidence interval.

Other covariates examined included sex, patient age, conditioning of the first SCT, use of methotrexate as GVHD prophylaxis, and numbers of selorogic HLA mismatch in graft-versus-host and host-versus-graft directions.

*Proportions of patients who achieved neutrophil engraftment.

†Standard risk included acute leukemia and non-Hodgkin lymphoma in any complete remission, CML in any chronic phase, and aplastic anemia. High risk included all other types of leukemia and non-Hodgkin lymphoma categories, and MDS-RAEB.

‡Grade of organ toxicities was evaluated by the CTCAE v3.0 [40].

§Alkylating agents included melphalan, busulfan, and cyclophosphamide. Other conditioning included fludarabine alone and a combination of fludarabine plus thiotepa or etoposide.

overcome immunologic rejection [21,26,45]. As previously reported in patients with aplastic anemia [19,46], the addition of 2-4 Gy TBI to the RIC regimen increased the probability of engraftment in a univariate analysis, although it did not have a significant effect in a multivariate analysis. In our preliminary data, 6 of the 10 patients who received second CBT without cytotoxic conditioning regimen (ie, ATG only, steroid only, etc.) experienced GF again after second SCT. Whereas the addition of alkylating agent and low-dose TBI to the conditioning regimen for the second RICBT enhanced neutrophil engraftment, it did not affect the overall outcomes in our study. To determine the best conditioning regimen for salvage RICBT after GF, further studies to evaluate regimens including fludarabine plus melphalan or cyclophosphamide with or without 2-4 Gy TBI will be required.

In our study, the TRM early after the second RICBT was extremely high (45% at day 100), mainly because of infectious complications, which was consistent with previous reports on CBT [5,17,29,30,47]. This is probably because of a prolonged period of severe neutropenia before and after the second RICBT in patients complicated with GF, which incubated carryover infections. To reduce the incidence of infection-related TRM, frequent monitoring and extensive treatment including granulocyte transfusion to support the intertransplant period may be needed [48]. Alternatively, the earlier application of RICBT while patients are still in better condition without infection may be preferred to reduce TRM.

When patients require a second SCT for GF, the selection of the donor source is critical. Based on the feasibility of second RICBT in our study, we suggest that CB carries the highest priority for selection because of its ready availability. Although the possibility of a second SCT or boost of stem cells from the same related donor of the first SCT has been reported [19,22], 75% of our patients had undergone CBT at the first transplant, which reflects the difficulty of finding a suitable donor. Another possibility is a second SCT from a haploidentical related donor [49,50]. The more rapid neutrophil engraftment after SCT using PBSC

**Table 5. Univariate and Multivariate Analysis of Overall Survival after the Second SCT**

| Covariates | Proportion at 1 Year (%) | Univariate P | Multivariate Hazard Ratio (95% CI) | Multivariate P |
|---|---|---|---|---|
| Disease risk at the first SCT* | | .03 | | .01 |
| Standard risk | 50 | | 1.00 | |
| High risk | 26 | | 2.14 (1.20-3.81) | |
| Type of graft failure | | .87 | | — |
| Primary | 36 | | — | |
| Secondary | 39 | | — | |
| Interval between the first SCT and second SCT | | .38 | | — |
| <50 days | 40 | | — | |
| ≥50 days | 31 | | — | |
| PS | | .2 | | — |
| 0-1 | 39 | | — | |
| 2-4 | 35 | | — | |
| Carryover organ toxicities at the second SCT† | | .001 | | .007 |
| Grade 0-2 | 41 | | 1.00 | |
| Grade 3-4 | 0 | | 2.84 (1.33-6.06) | |
| Carryover infection at the second SCT | | .14 | | — |
| Febrile neutropenia/none | 46 | | — | |
| Documented infection | 27 | | — | |
| Conditioning‡ | | .69 | | — |
| Alkylating agent–containing | 35 | | — | |
| Other | 40 | | — | |
| TBI | | .56 | | — |
| 2-4 Gy TBI | 37 | | — | |
| No TBI | 37 | | — | |
| TNC of the CB | | .77 | | — |
| $\geq 2.5 \times 10^7$/kg | 41 | | — | |
| $< 2.5 \times 10^7$/kg | 33 | | — | |

SCT indicates stem cell transplantation; PS, performance status; TBI, total-body irradiation; TNC, total nucleate cells; CB, cord blood; GVHD, graft-versus-host disease; HLA. human leukocyte antigen; CML, chronic myelogeneous leukemia; MDS, myelodysplatic syndrome; RAEB, refractory anemia with excess blasts; CI, confidence interval.

Other covariates examined included sex, patient age, conditioning of the first SCT, use of methotrexate as GVHD prophylaxis, and numbers of serological HLA mismatch in graft-versus-host and host-versus-graft directions.

*Standard risk included acute leukemia and non-Hodgkin lymphoma in any complete remission, CML in any chronic phase, and aplastic anemia. High risk included all other types of leukemia and non-Hodgkin lymphoma categories, and MDS-RAEB.

†Grade of organ toxicities was evaluated by the CTCAE v3.0. [40].

‡Alkylating agents included melphalan, busulfan, and cyclophosphamide. Other conditioning included fludarabine alone and a conbination of fludarabine plus thiotepa or etoposide.

from a haploidentical donor may decrease the risk of infectious complications in patients suffering from GF. However, compared to CBT, the feasibility of this procedure has not yet been established and the incidence of acute GVHD increases. In addition, collection of autologous stem cells prior to CBT might be an option to salvage a fraction of patients who experienced GF as previously reported [51]. Nevertheless, further studies are warranted to determine which types of transplant, CBT or SCT from a haploidentical related donor, can achieve better outcomes for patients suffering from GF.

This study has several inherent limitations. First, the patients and transplantation characteristics including the conditioning regimen, GVHD prophylaxis, and supportive care varied among the different centers. Second, the timing of and general conditions at the second RICBT differed among patients. Third, there may be unrecognized biases because only successful cases may have been collected. Finally, the duration of follow-up for patients in this study was too short to draw any definite conclusions. Nevertheless, the large cohort of 80 patients who received RICBT as salvage therapy for GF in the current study allowed us to make several clinically relevant observations.

In conclusion, we suggest that salvage therapy with a second RICBT is a feasible therapeutic option for patients who are suffering from GF. To achieve stable neutrophil engraftment after the second RICBT, conditioning with fludarabine plus alkylating agents and the infusion of CB containing $\geq 2.5 \times 10^7$/kg cells are preferable. A high TRM early after RICBT emphasizes the need for the earlier application of RICBT while patients still have better PS and have not yet acquired infection and organ toxicity. Prospective trials are needed to determine the ultimate utility of rescue RICBT using a fludarabine-based regimen including alkylating agents for patients suffering from GF.

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## AUTHORSHIP STATEMENT

F. Waki and T. Fukuda played a major role in designing and performing the research, verifying the integrity of and analyzing the data, and writing the manuscript. Y. Kanda played a major role in the statistical analyses and in developing the concept of the research. K. Masuoka, T. Yamashita, A. Wake, and S. Takahashi designed the research and contributed vital data to generate the final database. Y. Takaue and S. Taniguchi designed the research and contributed to writing or interpreting relevant parts of the manuscript. All other coauthors contributed vital data to generate the final database and interpreted relevant parts of the manuscript.

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## APPENDIX

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*Letter to the Editor*

# Virus-Associated Hemophagocytic Syndrome Caused by Pandemic Swine-Origin Influenza A (H1N1) in a Patient After Unrelated Bone Marrow Transplantation

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**Keywords:** virus-associated hemophagocytic syndrome, bone marrow transplantation, H1N1 influenza

## To the Editor

A 55-year-old Asian man was diagnosed as mantle cell lymphoma in 2004. He received conventional chemotherapy followed by autologous peripheral blood stem cell transplantation and achieved complete remission in 2004. The disease relapsed in 2007, and he underwent bone marrow transplantation from an unrelated donor after conditioning with fludarabine 30 mg/m$^2$ once daily i.v. for 5 days (total dose 150 mg/m$^2$) and cyclophosphamide 1 g/m$^2$ once daily i.v. for 2 days (total dose 2 g/m$^2$). He received tacrolimus and short-term methotrexate for graft-vs-host disease (GVHD) prophylaxis and achieved complete remission on day 89. On day 663, at the age of 61, he was referred to the emergency department of Nagoya University Hospital because of a 2-day history of fever and appetite loss. On initial evaluation, he was febrile (temperature : 38.5°C) with a pulse rate of 113 beats/min, blood pressure of 97/69 mmHg, respiratory rate of 20 breaths/min, and saturation of 88% at room air. He required 3 L of supplemental oxygen, which was supplied using a face mask, to maintain an oxygen saturation of 99% > A nasopharyngeal swab collected in the emergency department was negative for influenza A by rapid antigen testing. The patient had been immunized against seasonal influenza, but not against H1N1 influenza.

On arrival at the hematology-oncology department, lymphadenopathy or skin rash was not observed in the patient. He did not take any immunosuppressive agents, having no sign of chronic GVHD at that time. Initial laboratory findings showed a hemoglobin level of 12.4 g/dL, hematocrit of 35.9%, platelet count of 141,000/mm$^3$, and white blood cell (WBC) count of 13,200/mm$^3$ with an absolute neutrophil count of 6,700/mm$^3$. The chemistry profile showed that sodium, potassium, chloride, blood urea nitrogen, creatinine, glucose, asparate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were all within normal limits. The C-reactive protein (CRP) level was 7.6 mg/dL. Serum endotoxin and β-D glucan were negative. Serum immunoglobulin G, A, M level was 2,242, 27 and 156 mg/dL, respectively, and maintains the comparable level throughout the course. Soluble interleukin-2 receptor level was 1,360 U/mL. A chest radiograph was normal. A computed tomography scan of the chest was also normal. The patient showed no evidence of recurrence of lymphoma. A blood culture was obtained, and he received a dosage of intravenous imipenem/cilastatin (0.5 g×2/day).

On hospital day 9, the patient's clinical condition worsened with progressive dyspnea and hypoxia. The nasopharyngeal swab tested positive for influenza A by rapid antigen testing. A definite diagnosis was based on a positive result for pandemic H1N1 influenza virus by real-time reverse transcription-PCR (RT-PCR) for a nasopharyngeal swab. Oseltamivir (75 mg×2/day) was started on hospital day 9. Repeat laboratory data showed pancytopenia with a hemoglobin level of 9.3 g/dL, hematocrit of 28.1%, platelet count of

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29,000/mm$^3$, and WBC count of 3,700/mm$^3$ with an absolute neutrophil count of 1,700/mm$^3$. The chemistry profile revealed an AST of 140 IU/L, ALT of 69 IU/L, LDH of 626 IU/L, and ferritin level of 3,222 ng/mL. The CRP level was 3.3 mg/dL. Serum endotoxin and β-D glucan were negative. Coagulation profile revealed prothrombin time of 14.4 s, activated partial thromboplastin time of 42.4 sec, and fibrinogen of 422 mg/dL. The FDP and D-dimer level was normal. The triglyceride level was 101 mg/dL. A bone marrow aspiration biopsy revealed mature histiocytes exhibiting hemophagocytosis, suggesting a diagnosis of virus-associated hemophagocytic syndrome (VAHS) (Fig. 1). A high-resolution computed tomography (HRCT) scan of the chest revealed bilateral and peripheral focal ground-glass opacities in the upper, middle, and lower zones that are typical in H1N1 pneumonia (Fig. 2).[1,2] Multiple blood, urine, and sputum cultures were negative for bacteria and fungi. The patient's cytomegalovirus (CMV) antigenemia was negative. On hospital day 11, the repeat chemistry profile revealed an AST of 542 IU/L, ALT of 388 IU/L, LDH of 883 IU/L, and ferritin level of 8,814 ng/mL. As this patient met the criteria for the diagnosis of VAHS,[3] empiric treatment was initiated with 1 mg/kg of prednisolone on day 11, with subsequent reduction in fever. AST, ALT, and LDH levels became normal on hospital days 25, 36, and 25, respectively. The serum ferritin level decreased to 862 ng/mL on hospital day 22. The patient remained in a stable physical condition with a gradual dose reduction of prednisolone.

Pandemic (H1N1) influenza virus first appeared in March 2009 in Mexico and its rapid spread throughout the world marked the beginning of the first influenza pandemic in more than 40 years.[4,5] VAHS is an unusual disorder characterized by uncontrolled proliferation of mature histiocytes exhibiting hemophagocytosis.[3] A previous study demonstrated 6 of 68 (8.8%) cases of VAHS after allogeneic stem cell transplantation.[6] One case was infected with Epstein-Barr virus, 2 cases with CMV, and 3 cases showed no evidence of bacterial, fungal, or viral infections. Previous reports demonstrated VAHS associated with H3N2 seasonal influenza,[7,8] however, to the best of our knowledge, this patient represents the first report of VAHS associated with H1N1 influenza pneumonia. Physicians taking care of patients with hematologic malignancies should be aware of VAHS as an unusual but potentially severe and life-threatening complication of H1N1 influenza in immunosuppressed individuals. The most

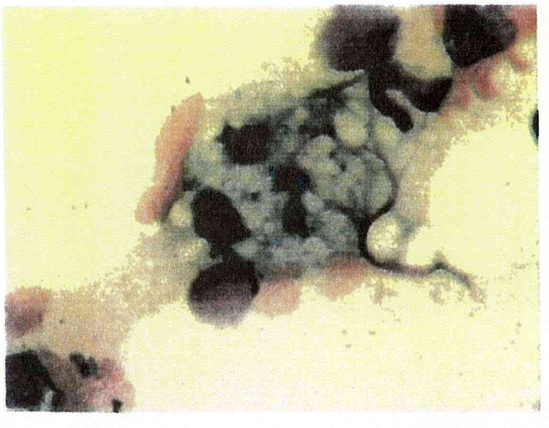

**Fig. 1.** Bone marrow with May-Giemsa staining showing hemophagocytosis of an erythrocyte and platelets

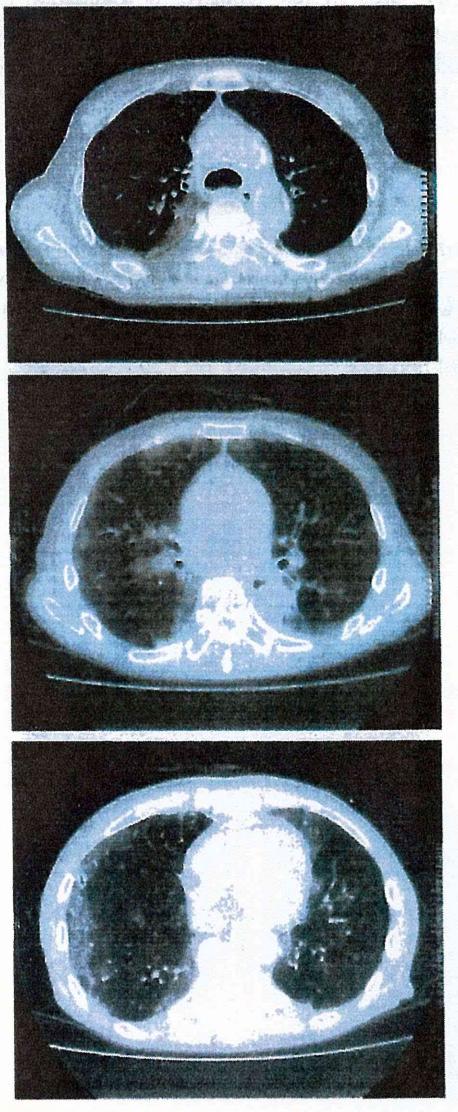

**Fig. 2.** 61-year-old male patient with H 1 N 1 pneumonia. HRCT scan of the chest obtained on hospital day 11 showing bilateral and peripheral focal ground-glass opacities in the upper (*A*), middle (*B*), and lower (*C*) zones.

common HRCT manifestations of H1N1 virus-associated pneumonia are bilateral ground-glass opacities and/or bilateral areas of consolidation, which showed a more severe clinical course.[1] Early computed tomography may help clinicians recognize incipient cases of H1N1 virus-associated pneumonia.

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ORIGINAL ARTICLE

# Outcome of medium-dose VP-16/CY/TBI superior to CY/TBI as a conditioning regimen for allogeneic stem cell transplantation in adult patients with acute lymphoblastic leukemia

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**Abstract** The choice of conditioning regimen before allogeneic stem cell transplantation (SCT) in patients with acute lymphoblastic leukemia (ALL) is important. We retrospectively compared outcomes of medium-dose VP-16/cyclophosphamide/total body irradiation (VP/CY/TBI) regimen and CY/TBI. Five hundred and twenty-nine patients (VP/CY/TBI: $n = 35$, CY/TBI: $n = 494$) who met all of the following criteria were compared: first time for SCT, aged 15–59 years; first or second complete remission at SCT; bone marrow or peripheral blood as stem cell source; and HLA phenotypically matched donor. Median age of the patients was 34 years, and patients who received VP/CY/TBI were younger (28 vs. 34 years, $P = 0.02$). Cumulative incidences of relapse and non-relapse mortality (NRM) were higher for patients who

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