Pre-engraftment syndrome (PES) occurring after cord blood transplantation (CBT) is poorly characterized. We reviewed 52 consecutive double-unit CBT recipients treated for high-risk hematologic malignancies. PES was defined as unexplained fever >38.3 °C (101.0 °F) not associated with infection and unresponsive to antimicrobials, and/or unexplained rash occurring before or at neutrophil recovery. CBT recipients (median age, 38 years; range, 3-66 years) received either myeloablative (MA; n = 36) or nonmyeloablative (NMA; n = 16) conditioning. Sixteen patients (31%) fulfilled PES criteria: 15 with fever (median onset, 39 °C [102.2 °F]), 13 of whom also had rash, and 1 with rash alone. The median onset was 9 days (range, 5-12 days) posttransplantation (a median of 14 days before neutrophil recovery). Sixteen patients (14 with PES and 2 with infection and possible PES) received intravenous methylprednisolone (median dose, 1 mg/kg; median duration, 3 days); 15 (94%) experienced resolution of fever within 24 hours. Recurrent PES (n = 3) resolved with retreatment. There was no association between the development of PES and the likelihood of sustained donor engraftment, speed of neutrophil recovery, grade II-IV acute graft-versus-host disease (aGVHD), day-180 treatment-related mortality (TRM), or survival. PES is common after CBT, precedes neutrophil recovery, is distinct from and does not predict for aGVHD, and responds promptly to short-course corticosteroid therapy.


KEY WORDS: Hematopoietic stem cell transplantation, Cord blood transplantation, Engraftment, Graft-versus-host disease

INTRODUCTION

Engraftment syndrome, a clinical entity of unknown pathogenesis, has been described in patients receiving both autologous [1-3] and allogeneic [4,5] hematopoietic stem cell transplantation (HSCT). Whereas a uniform definition is lacking, one definition, suggested by Spitzer [6], is a clinical syndrome after HSCT characterized by noninfectious fever, erythematous skin rash, and pulmonary infiltrates occurring immediately before or at neutrophil engraftment. Kishi et al. [7] were the first to report a pre-engraftment immune reaction, which occurred in 35 of 45 (78%) adult recipients of reduced-intensity conditioning (RIC) cord blood transplantation (CBT). This was associated with various manifestations, including fever, rash, diarrhea, jaundice, and weight gain >10% from baseline occurring before neutrophil engraftment and not explained by infection or adverse drug reactions. The authors suggested that this pre-engraftment syndrome (PES) differs from engraftment syndrome or acute graft-versus-host disease (aGVHD) [7]. PES remains poorly characterized, however, and the prognosis and appropriate management are unclear. We conducted a retrospective review of 52 consecutive CBT recipients treated for high-risk hematologic malignancies to determine the incidence, manifestations, and outcomes of PES. Our hypothesis was that PES is distinct from and does not predict aGVHD.

MATERIALS AND METHODS

Patient and Graft Characteristics

This was a retrospective review of 52 consecutive CBT recipients who received a first allograft at
Memorial Sloan-Kettering Cancer Center. Collection of transplantation complication and outcome data was sanctioned by the Center’s Institutional Review Board. Survivors had at least 100 days of follow-up posttransplantation. The patients had a median age of 38 years (range, 3-66 years) and a median weight of 70 kg (range, 13-102 kg), and all had a high-risk hematologic malignancy: acute myelogenous leukemia (AML; n = 12), acute lymphoblastic leukemia (ALL; n = 10), acute biphenotypic leukemia (n = 2), non-Hodgkin lymphoma (NHL; n = 15), Hodgkin lymphoma (HL; n = 9), chronic lymphocytic leukemia (CLL; n = 3), or prolymphocytic leukemia (n = 1). Patients underwent CBT after receiving either myeloablative (MA; n = 36) or nonmyeloablative (NMA; n = 16) conditioning according to age, extent of previous therapy, comorbidities, and diagnosis. Cyclosporine-A (CsA) and mycophenolate mofetil (MMF) were used for GVHD prophylaxis, and all patients received posttransplantation granulocyte colony-stimulating factor (G-CSF). All patients received double-unit grafts to augment engraftment [8,9], with a median infused total nucleated cell (TNC) dose of 2.5 × 10^7/kg (range, 1.42-7.30 × 10^7/kg) in the larger unit and 1.9 × 10^7/kg (range, 0.91-5.26 × 10^7/kg) in the smaller unit. Units were 6/6 (n = 5), 5/6 (n = 51), and 4/6 (n = 48) HLA-A, -B antigen, and -DRB1 allele–matched to the recipient, respectively. Donor–recipient and unit–unit HLA matching also were determined at high resolution for HLA-A, -B, -C, -DRB1, and -DQ alleles.

All patients or their parents signed informed consent before transplantation. Patients were hospitalized in high-efficiency particulate air (HEPA)-filtered single protective environment rooms and received prophylaxis for fungal infections (including mold), herpes simplex, and Pneumocystis jiroveci, as well as bacterial infections during neutropenia. Neutropenic fever was treated with broad-spectrum intravenous (i.v.) antibiotics.

Definition of PES

Medical records were reviewed for clinical features suggestive of PES and the associated laboratory and radiologic findings. PES was defined as unexplained fever > 38.3 °C (101°F) not associated with documented infection and unresponsive to antimicrobial manipulations, and/or unexplained erythematous skin rash resembling that of aGVHD, with either the fever or the rash occurring before or at neutrophil recovery. Specifically, fever attributed to PES was not associated with any clinical evidence of infection, with patients having both a negative infectious disease workup and a continued lack of response to broad-spectrum antimicrobial agents. All patients with fever underwent an extensive infectious disease workup that included serial blood cultures (all ports), urine cultures, stool studies (if diarrhea was present), relevant viral polymerase chain reaction (PCR) findings, and relevant radiologic findings, including lung computed tomography scan. The erythematous skin rash attributed to PES was not associated with any clinical suspicion of drug allergy.

Weight gain was calculated as the percent change in weight between the day of CBT and the onset of PES. For comparison, the weight gain in patients with no evidence of PES was calculated as the percent change in weight between the day of CBT and day 9 posttransplantation (ie, the median day of onset of PES). Noninfectious diarrhea was defined as passage of liquid stools more than twice a day for at least 3 consecutive days with stool studies negative for any infectious etiology.

Statistical Analysis

Because there were no early deaths (before day 28 posttransplantation), there were no competing risks in the calculation of PES incidence. Time to neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 0.5 × 10^9/L after the first posttransplantation nadir. Sustained donor engraftment was defined as sustained donor-derived count recovery with donor chimerism of at least 90% (both units combined). Overall staging of aGVHD was based on International Bone Marrow Transplant Registry criteria [10]. Treatment-related mortality (TRM) was defined as any death not from relapse or persistence of malignancy. Neutrophil engraftment, acute GVHD (aGVHD), and TRM were computed using the cumulative incidence function. For neutrophil engraftment, the competing risks were autologous recovery, infusion of a backup graft, or death. Graft failure or death was the competing event for aGVHD, whereas relapse was the competing event for TRM. Survival was calculated using Kaplan-Meier methodology. The relationships between PES outcome and binary, ordinal, and continuous factors were determined using Fisher’s exact test, Wilcoxon’s rank-sum test, and the t-test, respectively. The difference in survival rates based on PES classification was determined using the log-rank test; the difference in the cumulative incidence curves was based on Gray’s test.

RESULTS

Incidence and Manifestations of PES

Of the 52 patients eligible for analysis, 16 (31%), including 12 recipients of MA conditioning and 4 recipients of NMA conditioning, fulfilled the diagnostic criteria for PES. Of these 16 patients, 15 (94%) had unexplained fever, 13 of whom also had rash. The median temperature at onset was 39 °C (102.2°F) (range, 38.4-39.4 °C), and fever exhibited a spiking pattern.
One patient (a recipient of NMA conditioning) had rash alone as the sole manifestation of PES. Although we originally defined PES as potentially occurring before or at neutrophil recovery, we found that the median day of onset of PES was early, at 9 days posttransplantation (range, 5-12 days). This was a median of 14 days before neutrophil recovery overall. The median total white cell count at PES onset was \(0 \times 10^9/L\) (range, \(0-0.9 \times 10^9/L\)), with a median ANC of \(0 \times 10^7/L\) (range, \(0-0.5 \times 10^7/L\)). The day of onset and appearance of symptoms were similar regardless of conditioning.

The remaining 36 patients who did not fulfill strict PES criteria included 5 patients without posttransplantation fever or rash, 26 patients with fever secondary to either documented infection or febrile neutropenia responsive to antimicrobials, and 5 patients with fever judged by the treating physician to be due to infection and possible PES.

The mean weight gain at PES onset was 3% in both the PES patients and non-PES patients at the same time point posttransplantation (\(P = .60\)). In addition, almost half of the patients in each group had noninfectious diarrhea (\(P = 1.00\)). There was no significant difference in mean peak bilirubin level between the 2 groups at days 0-7 (\(P = .32\)), days 8-15 (\(P = .29\)), days 16-21 (\(P = .76\)), or days 22-28 (\(P = .54\)) posttransplantation. Notably, 11 of the 16 PES patients (69%) developed hypoxia and/or pulmonary infiltrates at a median of 12 days (range, 7-15 days) posttransplantation, compared with 16 of 36 non-PES patients (44%) at a median of 12 days posttransplantation (range, 5-32 days). This difference was not statistically significant, however (\(P = .14\)).

Human herpesvirus 6 (HHV-6) reactivation may be associated with fever and rash and is well documented after CBT [11]. Thus, HHV-6 viremia was examined as a potential factor accounting for the manifestations of PES. Fifteen of the 16 PES patients underwent serial assays for HHV-6 virus reactivation using quantitative PCR of serum, and all were positive. However, of the 26 patients without PES evaluated, 24 (92%) also had HHV-6 reactivation. In patients with PES, the mean time to first detection of HHV-6 viremia (>100 copies/mL) was 24 days posttransplantation (range, 10-37 days), notably later than the onset of PES. This was not different from the mean time to detection of 21 days in the non-PES patients (range, 10-40 days; \(P = .25\)). The mean peak HHV-6 load was 22,900 copies in the PES patients (range, 200-116,000 copies) and 17,100 copies in the non-PES patients (range, 100-128,000 copies; \(P = .58\)). Thus, we found no evidence suggesting that the manifestations of PES can be accounted for by HHV-6.

### Response to Corticosteroids

A total of 16 patients (14 with PES and 2 with infection and possible PES) received i.v. methylprednisolone (MP) to treat PES. The treated patients had high fever for a median of 5.5 days (range, 3-11 days) before corticosteroid treatment and received a median dose of 1 mg/kg (range, 0.5-2 mg/kg). All patients treated with MP responded, as evidenced by resolution of fever within 48 hours along with resolution of rash. After the first dose of MP, fever resolved within 12 hours in 13 of the 16 patients (81%), within 13-24 hours in 2 patients, and within 25-48 hours in 1 patient. Two remaining PES patients did not receive MP. One of these patients had rash alone and experienced spontaneous resolution within 7 days; the other was not treated because of concerns about the increased risk of infection with corticosteroid therapy and remained febrile for 33 days. Corticosteroid treatment was

### Table 1. Comparison of Patient and Graft Characteristics in PES Patients and Non-PES Patients

<table>
<thead>
<tr>
<th></th>
<th>PES (n = 16)</th>
<th>Non-PES (n = 36)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (range)</td>
<td>31 (3-65)</td>
<td>41 (7-63)</td>
<td>.08</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>20</td>
<td>.77</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Weight, kg, median (range)</td>
<td>66 (13-108)</td>
<td>71 (22-109)</td>
<td>.44</td>
</tr>
<tr>
<td>Diagnosis, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid malignancy</td>
<td>4</td>
<td>10</td>
<td>1.00</td>
</tr>
<tr>
<td>Myeloid malignancy</td>
<td>12</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Preparative regimen, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloablative</td>
<td>12</td>
<td>24</td>
<td>.75</td>
</tr>
<tr>
<td>Nonmyeloablative</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Infused TNC (\times 10^7/kg), mean (range)</td>
<td>4.3 (2.7-12.6)</td>
<td>4.8 (2.6-9.6)</td>
<td>.53</td>
</tr>
<tr>
<td>Donor–recipient HLA match, median (range)*</td>
<td>6/10 (4-8/10)</td>
<td>6/10 (3-9/10)</td>
<td>.46</td>
</tr>
<tr>
<td>Match of better-matched unit</td>
<td>5/10 (4-8/10)</td>
<td>5/10 (2-9/10)</td>
<td>.02</td>
</tr>
<tr>
<td>Match of lesser-matched unit</td>
<td>6/10 (3-10/10)</td>
<td>5/10 (2-9/10)</td>
<td>0.57</td>
</tr>
<tr>
<td>Unit–unit HLA match, median (range)*</td>
<td>2.3 (1.3-5.3)</td>
<td>2.5 (1.4-5.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>Engrafting unit TNC (\times 10^7/kg), mean (range)†</td>
<td>6/10 (4-8/10)</td>
<td>6/10 (2-8/10)</td>
<td>0.90</td>
</tr>
<tr>
<td>Donor–recipient HLA match of engrafting unit, median (range)†</td>
<td>6/10 (4-8/10)</td>
<td>6/10 (2-8/10)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

PES indicates pre-engraftment syndrome; TNC, total nucleated cell.

*Ten allele HLA-A, -B, -C, -DRB1, and -DQ match.

†Excludes 2 patients with graft failure in each group and the single patient who was in the no-PES group who engrafted with both units.
continued for a median of 3 days (range, 2–44 days). Of the 16 treated patients, 3 had recurrent fever attributed to PES, which resolved with corticosteroid retreatment (median duration, 10 days; range, 1–27 days).

**Patient Demographics and Graft Characteristics, and the Development of PES**

Table 1 compares patient demographics and graft characteristics of the 16 PES patients and the 36 non-PES patients. There were no significant differences between the 2 groups in terms of age, sex, weight, underlying malignancy, or conditioning regimen. There also were no between-group differences in total infused TNC dose, donor–recipient HLA matching of each CB unit, the unit–unit HLA match, the infused TNC dose of the engrafting unit, or the donor–recipient HLA match of the engrafting unit (at low or high resolution).

**PES and Transplantation Outcome**

Overall, 3 patients experienced primary graft failure, and 1 patient experienced secondary graft failure. Three of these patients received MA conditioning, and 1 patient received NMA conditioning. Thus, for the entire study group the cumulative incidence of sustained donor engraftment was 92% (95% confidence interval [CI] = 84%-100%), with neutrophil recovery occurring at a median of 25 days in MA recipients (range, 13–43 days) and 11 days (range, 7–36 days) in NMA recipients. Consistent with previous reports, engraftment was accounted for by a single unit, except in 1 patient who had sustained engraftment of both units.

There was no difference in sustained donor engraftment between patients with and without PES, with graft failure occurring in 2 PES patients and 2 non-PES patients ($P = .58$). Excluding the 4 patients who experienced sustained donor engraftment, in patients who received MA conditioning, the median time to neutrophil recovery was 25 days (range, 13–43 days) in PES patients and also 25 days (range, 14–33 days) in non-PES patients ($P = .83$). In the patients who received NMA conditioning, these medians were 22 days (range, 7–36 days) in PES patients and 11 days (range, 7–22 days) in non-PES patients ($P = .35$).

For the entire study group, the cumulative incidence of day-100 grade II–IV aGVHD was 40% (95% CI = 27%-53%). There was no difference in aGVHD incidence between PES patients and non-PES patients (44% vs 39%; $P = .79$) (Figure 1). The median time of onset of aGVHD was 50 days (range, 34–70 days) in the PES patients and 41 days (range, 29–99 days) in the non-PES patients.

For the entire group, TRM at day 180 was 24% (95% CI = 13%–35%). Four PES patients and 4 non-PES patients died from transplantation-related causes by day 180 ($P = .23$). With a median follow-up of 12 months (range 1–36 months), the 1-year overall survival (OS) was 64% (95% CI = 52%–80%). There was no difference in OS between PES patients and non-PES patients (61% vs 62%; $P = .43$) (Figure 2).

**DISCUSSION**

PES has been well described after autologous transplantation, although the reported incidence is highly variable depending on the definition used [6]. ES after allogeneic transplantation is less well understood, with some investigators attributing it to hyperacute aGVHD [6]. Interestingly, a number of early reports on CBT described an onset of aGVHD well in advance of neutrophil engraftment. For example, Sanz et al. [12] described a median time to onset of aGVHD of 9 days (range, 4–14 days) with a median time to neutrophil recovery of 22 days (range, 13–52 days) [12]. Furthermore, while Wagner et al. [13] reported a median onset of aGVHD of 35 days.
posttransplantation, the lower limit of the range was 8 days posttransplantation. It is likely that some patients with this “early aGVHD” may have had PES. Kishi et al. [7] defined a pre-engraftment immune reaction in reduced-intensity single-unit CBT recipients that included the presence of fever, skin eruption, diarrhea, jaundice, and weight gain > 10% of baseline that could not be attributed to infection or adverse effects of medications. These broad criteria likely accounted for the high incidence of 78% of PES reported in their study. Using a stricter definition of unexplained noninfectious fever and/or unexplained skin rash, we found that 31% of our CBT patients fulfilled the criteria for PES. This indicates that this syndrome, as with allogeneic transplantation using other stem cell sources [6], is relatively common. On the other hand, our strict definition may have led to an underestimation of this syndrome. We excluded 5 patients who were judged by the treating transplantation physician to be infected; these patients also could have had PES (especially because 2 of them were treated with and responded to corticosteroids).

The onset of PES in our CBT series was identical to that described by Kishi et al. [7], a median of 2 weeks before neutrophil recovery, clearly justifying the term “pre-engraftment.” Interestingly, we found that weight gain, hyperbilirubinemia, and noninfectious diarrhea were no more frequent in the PES patients than in the non-PES patients. The difference between the 2 groups in terms of hypoxia and/or pulmonary infiltrates also was not significant. Nonetheless, the temporal correlation with the development of pulmonary manifestations following the onset of unexplained fever in patients with PES merits further study. Such investigation may be hampered by our incomplete knowledge of the etiology or predisposing factors to the syndrome, however.

The development of PES in autologous and allogeneic BMT recipients has been associated with a wide variety of risk factors. In studies of autologous transplantation, predisposing factors included specific diagnoses [3,14], less extensive previous therapy [14], busulfan (Bu)-based conditioning [2], a greater number of infused hematopoietic cells [2,15], use of G-CSF [1], and early and steep neutrophil recovery [2]. In the allogeneic setting, Gorak et al. [4] described older age, female sex, and the use of amphotericin formulations as predisposing factors for PES after NMA conditioning. Schmid et al. [5] also reported treatment with amphotericin, use of G-CSF, and grafts with higher cell doses as risk factors for PES in pediatric allograft recipients. In contrast, we found no significant differences between patients with and without PES in terms of age, sex, conditioning regimen, infused cell dose, or HLA match. We also found that PES could not be attributed to HHV6 virus reactivation. Thus, the mechanism and predisposing factors for this relatively common syndrome after CBT remain unknown.

Although corticosteroids have been used to treat ES after autologous HSCT [1-3,14] or allogeneic HSCT [4,5] as well as pre-engraftment immune reactions in CBT recipients [7,16], there is no agreement about the correct dose or treatment duration. All of the PES patients in this study treated with corticosteroids responded rapidly, with most fevers resolving within 12 hours. It is interesting to postulate that PES may have become more frequent since the abandonment of corticosteroids as GVHD prophylaxis by many centers. But, this does not warrant the use of either corticosteroids (with their associated infection risk) or methotrexate (with its associated risk of delayed engraftment) [16] as preventative therapy for PES after CBT, given that this syndrome is profoundly steroid-sensitive. Furthermore, although the majority of patients did not experience recurrent PES, the 3 patients who did so responded promptly to retreatment. More importantly, there was no association between PES and the subsequent development of aGVHD, with a median day of aGVHD onset of 50 days posttransplantation in PES patients. Clearly, a detailed workup to exclude infection is mandatory in CBT recipients being considered for PES. However, for those patients meeting the criteria for PES (pre-engraftment, without documented infection, no response to broad antimicrobial coverage, and no other features such as progressive gut or liver pathology, suggesting aGVHD), a reasonable approach is to diagnose PES and provide treatment with short-course corticosteroids, and not diagnose early aGVHD predating engraftment. Although the optimal therapy for PES is unknown, and no definitive recommendations can be made based on a relatively small series, we are now investigating 1 mg/kg/day of i.v. methylprednisolone for 3 days with no taper and close monitoring for patient well being after corticosteroid cessation.

One further aspect of PES after CBT deserves emphasis. Whereas we did not find increased mortality in our PES patients, PES is associated with significant morbidity. Failure to recognize this syndrome in CBT recipients risks unnecessary complications of high fevers with possible pulmonary complications, as have been seen in autologous transplant recipients with PES. Prompt recognition of PES and treatment with a short-course corticosteroid regimen also can help avoid unnecessarily long, empiric courses of treatment that could promote opportunistic infections. Thus, further validation of our findings in a prospective investigation of a larger series of CBT recipients is warranted. This should include studying the incidence of PES to ascertain whether there are differences between recipients of single-unit and double-unit CBT,
characterizing associated end-organ toxicities (especially possible pulmonary manifestations), and, most importantly, searching for biomarkers that may provide clues to etiology [17].

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**REFERENCES**