

## Autografting

# Engraftment syndrome after autologous peripheral blood progenitor cell transplantation in pediatric patients: a prospective evaluation of risk factors and outcome

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### Summary:

**We prospectively analyzed the incidence, risk factors and outcome of engraftment syndrome (ES) in 112 patients undergoing autologous peripheral blood progenitor cell transplantation with different malignancies between January 1999 and December 2003. The median age was 8 years (range 1–18). There were 73 males. There were 37 hematological neoplasias and 75 solid tumors. Disease status at transplantation was early in 49, intermediate in 15 and 48 in advanced phase. The median CD34+ cells infused was  $4.6 \times 10^6/\text{kg}$ . With a median follow-up of 23 months (1–116 months), 38 patients developed ES. The cumulative incidence of ES was  $34.5 \pm 4.5\%$  and the event-free survival was  $58.3 \pm 12\%$ . There were no differences in the causes of death between patients with or without ES. A high number of CD34+ cells/kg infused, patients transplanted in early phase, the type of malignancy (solid tumor) and conditioning regimens other than busulfan based were significantly associated with ES in a multivariate analysis.**

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colony-stimulating factor (G-CSF),<sup>5</sup> lack of sepsis in the first week of neutropenia,<sup>5</sup> high numbers of CD34+ cells,<sup>3</sup> conditioning with busulfan (BU),<sup>3</sup> high mononuclear cell dose<sup>2</sup> and breast cancer.<sup>2</sup> We also retrospectively analyzed risk factors for ES and found that the mobilization regimen used was the only variable significantly associated with ES: patients mobilized with high doses of G-CSF in steady-state hematopoiesis had a higher incidence of ES.<sup>1</sup> In order to gain more information in this setting of patients regarding this post transplant event, we prospectively analyzed the incidence, risk factors and clinical outcome of ES in pediatric patients undergoing autologous PBPC.

### Patients and methods

#### *Design of the study*

This study was designed to evaluate prospectively the incidence, risk factors and outcome of ES in a cohort of pediatric patients using the same mobilization regimen. The primary end points were time to ES, cause of death and event-free survival (EFS). The secondary end points were time to neutrophil and platelet engraftment, number of hospitalization days, supportive care and costs calculated as previously reported.<sup>6</sup>

#### *Patients characteristics*

From January 1999 to December 2003, 112 autologous peripheral blood progenitor cell transplants for hematological malignancies and solid tumors were consecutively performed at hospital Niño Jesús. The main patient characteristics are shown in Table 1. Parents informed consent was obtained in all cases.

#### *Mobilization and apheresis*

All patients were mobilized by the administration of G-CSF (Neupogen; Amgen, Thousand Oaks, CA, USA) at a dose of  $12 \mu\text{g}/\text{kg}$  twice daily subcutaneously for 4 consecutive days.<sup>7</sup> On day +5 after mobilization, PBPC collections were performed using a Cobe Spectra cell separator (Cobe, Lakewood, CO, USA) by large-volume leukapheresis through a central venous catheter. Details of apheresis procedures have been previously reported.<sup>8</sup>

Engraftment syndrome (ES) has emerged as one of the most important causes of early morbidity and nonrelapse mortality in children undergoing autologous peripheral blood progenitor cell transplantation (PBPC).<sup>1</sup> However, the reported incidence has varied widely in part due to the lack of uniform diagnostic criteria.<sup>2–4</sup> This has also influenced the analysis of risk factors for ES. Several retrospective studies have described a variable numbers of risk factors for ES such as postinfusion use of granulocyte

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**Table 1** Patients characteristics (n = 112)

Age: median (range)	8 (1–18)
Gender: male/female	73/39
<i>Diagnosis</i>	
ALL	10
AML	13
NHL	9
HD	5
CNST	19
PNET/ES	19
NB	17
RB	5
WT	4
Osteosarcoma	9
Germinal tumor	2
<i>Disease status at transplantation</i>	
Early	49
Intermediate	15
Advanced	48
<i>Conditioning</i>	
With BU	89
Without BU	23
CD34+ cells × 10 <sup>6</sup> /kg: median (range)	4.6 (1–50)
<i>G-CSF postinfusion</i>	
Yes/no	46/66

ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; NHL = non-Hodgkin's lymphoma; HD = Hodgkin's disease; CNST = central nervous system tumor; PNET = primitive neuroectodermal tumor; ES = Ewing's sarcoma; NB = neuroblastoma; RB = rhabdomyosarcoma; WT = Wilms' tumor; BU = busulfan; CR = complete remission; PR = partial relapse; PD = progressive disease; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor.

#### Conditioning regimen and supportive care

Preparatory regimens were different according to the underlying disease and the children's age. These were busulfan (BU) plus melphalan (ME) in 42 patients, BU plus cyclophosphamide (CY) in 22 patients, carboplatin plus etoposide in 16 patients, BU plus ME plus topotecan in eight patients, BU plus CY plus etoposide in seven patients, BU plus ME plus thiotepa in six patients, BU plus thiotepa in four patients, total body irradiation (TBI) plus CY in four patients and carmustine plus CY plus etoposide in three patients.

The transplant procedures were performed in single rooms with HEPA-filtered air. Infectious prophylaxis included cotrimoxazole from conditioning beginning until day -1. When the regimen included BU, clonazepam and heparin were added for prophylaxis of seizures and veno-occlusive disease, respectively. On day 0, collected cells were infused. A set of patients received G-CSF postinfusion at a dose of 10 µg/kg/day until neutrophil engraftment as part of a prospective study to assess the clinical and economic impact of using postinfusion G-CSF. Platelets were transfused if the platelet counts decreased below 10 × 10<sup>9</sup>/l or in cases of bleeding, and red blood cells were given to maintain a hemoglobin level > 8 g/dl. Febrile neutropenia was treated with broad-spectrum empiric antibiotic therapy. Amphotericin B was given for persistent fever after

3–5 days. Patients unable to maintain an adequate oral caloric intake were fed with parenteral nutrition.

All patients who developed ES received corticosteroids at high doses (500 mg/m<sup>2</sup>/day in two doses) for 3 days, followed by tapering off as soon as possible. Discharge criteria included neutrophil engraftment, adequate oral intake and absence of infections.

#### Definitions

ES was defined as the presence of fever, skin rash and noncardiogenic pulmonary edema (based on clinical and radiologic criteria) during the periengraftment period when all microbiological studies were negative.<sup>4,5,9</sup> Disease status was classified as follows: early disease including acute leukemia, lymphoma and solid tumor in first remission, intermediate disease as any patient in second remission and advanced disease as beyond second remission, relapse or persistent disease. Neutrophil engraftment was defined as days to ANC > 0.5 × 10<sup>9</sup>/l for 3 consecutive days. Platelet engraftment was defined as the time to achieve > 20 × 10<sup>9</sup> platelets/l for 3 days without transfusion. Transplant-related mortality (TRM) was defined as any cause of death other than relapse.

#### Statistical analysis

Data are expressed as median and range. The impact of demographic and transplant-related variables on ES was analyzed by means of a proportional hazard regression model.<sup>10</sup> The probabilities of developing ES and the EFS were estimated by the Kaplan–Meier method.<sup>11</sup> Survival curves comparison was performed using the log-rank test. Results were considered significant if the *P*-value was < 0.05.

## Results

#### Incidence of engraftment syndrome

All patients engrafted. Neutrophil and platelet engraftments were achieved at a median time of 10 days (range 7–21) and 12 days (range 6–91), respectively. Out of 112 patients, 38 (34%) developed ES. With a median follow-up of 23 months (1–116 months), the cumulative incidence of ES was 34.5 ± 4.5%. Pretransplant characteristics of these patients are described in Table 2. There was no clinical or bacteriological evidence of infection in any case. A bronchoscopy with bronchoalveolar lavage was performed in three patients. Diffuse alveolar hemorrhage was excluded and microbiological studies were negative in all cases. The median time to onset was day +9 (range 4–19). The clinical manifestations of ES in all patients are shown in Table 3.

#### Risk factors for engraftment syndrome

Variables significantly associated to ES in univariate analysis were disease status at transplantation, number of CD34+ cells/kg infused, a diagnosis other than acute myeloblastic leukemia (AML) and neuroblastoma (NB), while a trend toward a higher incidence of ES was observed

**Table 2** Clinical characteristics of patients with ES (*n* = 38)

Age: median (range)	8 (1–18)
Gender: male/female	22/16
<i>Diagnosis</i>	
ALL	5
AML	2
NHL	1
HD	1
CNST	8
PNET/ES	7
NB	3
RB	3
WT	1
Other	7
<i>Disease status at transplantation</i>	
Early	19
Intermediate	5
Advanced	14
<i>Conditioning</i>	
With BU	29
Without Bu	9
CD34+ cells × 10 <sup>6</sup> /kg: median (range)	4.5 (1.4–50)
<i>G-CSF postinfusion</i>	
Yes/no	14/24

For abbreviations, see footnote to Table 1.

**Table 3** Clinical manifestations of ES (*n* = 38)

<i>Clinical components</i>	<i>Patients (no. (%))</i>
Fever	38 (100)
Skin rash	38 (100)
Pulmonary edema	29 (76)
Hepatic dysfunction	4 (10.5)
Renal insufficiency	3 (7.8)
Encephalopathy	2 (5.2)

**Table 4** Univariate analysis of risk factors of ES

<i>Variable</i>	<i>Exp</i>	<i>HR</i>	<i>95% CI</i>	<i>P-value</i>
Age	0.01	1.02	0.96–1.08	0.4
Gender	−0.37	0.69	0.36–1.31	0.26
<i>Diagnosis</i>				
AML	−1.68	0.18	0.03–0.89	0.03
NHL	−1.96	0.13	0.01–1.11	0.06
NB	−1.52	0.21	0.05–0.84	0.02
BU conditioning	−0.60	0.54	0.28–1.03	0.06
Early phase of disease	0.35	1.41	0.71–2.82	0.02
G-CSF postinfusion	−0.13	0.87	0.45–1.69	0.69
CD34+ /kg	0.039	1.04	1.004–1.07	0.03

For abbreviations, see footnote to Table 1.

in patients conditioned with other than a BU-based regimen (Table 4). Age, gender and the use of G-CSF postinfusion were the variables not significantly associated with the development of ES. There were 12 patients who needed amphotericin B but no influence was found on the incidence of ES.

**Table 5** Multivariate analysis of risk factors of ES

<i>Variable</i>	<i>Exp</i>	<i>HR</i>	<i>95% CI</i>	<i>P-value</i>
Hematologic tumor	−0.85	0.42	0.19–0.94	0.03
BU-based conditioning	−0.73	0.47	0.24–0.92	0.02
Early status of disease	0.75	2.13	1.01–4.45	0.04
CD34+ /kg	0.032	1.032	1–1.06	0.04

A high number of CD34+ cells/kg, the type of malignancy (solid tumor), the conditioning regimen (other than BU-based) and early phase of the underlying disease were associated with ES in multivariate analysis (Table 5).

### Clinical outcome and causes of death

The median length of ES was 5 days (range 2–14). Patients who developed ES had longer hospitalization stay, more transfusion requirements and supportive care with parenteral nutrition, morphine and inotropic drugs. Three patients needed admission to intensive care unit (ICU) for mechanical ventilation (MV) and the median time on MV was 37 days (range 25–57). The median time on corticosteroids therapy was 5 days (range 4–14). In addition to more supportive care, there was a trend toward higher costs in the group of patients who developed ES. More detailed data are shown in Table 6. The causes of death are shown in Table 7. The main cause of death in both groups was relapsing disease. In all, 29 patients died: 10 in the group with ES and 19 in the patients without ES. TRM and EFS was 6.1 ± 4.2 and 58.3 ± 12% in the group with ES and 4.8 ± 2.7 and 55.2 ± 9.5% in the patients without ES, respectively (*P* = NS).

### Discussion

We previously reported that ES was the main cause of morbidity and mortality, other than relapsing disease, following autologous PBPCT in children.<sup>1</sup> We now present here the largest prospective study of ES after autologous PBPCT in pediatric patients. In 1995, Lee *et al*<sup>5</sup> described ES as the presence of skin rash and noninfectious neutropenic fever in the periengraftment period. Several reports followed, but the incidence and clinical presentation widely varied due to the lack of standard criteria for the diagnosis of ES. The majority of these reports were based on adult patients, with only a few cases of children. Spitzer *et al*<sup>4</sup> established more restrictive major (fever, skin rash and noncardiogenic pulmonary edema) and minor (hepatic dysfunction, renal insufficiency, weight gain >2.5% and transient encephalopathy) diagnostic criteria that should be present within 96 h of the engraftment. We have followed these criteria in this study.

Several risk factors have been associated with ES. We found that ES in pediatric patients was associated with high numbers of CD34+ cells, with early phase of the underlying disease and with solid tumors, factors already reported in adult patients.<sup>2,3,12,13</sup> Conditioning regimen has been also implicated in the development of ES. Ravoet *et al*<sup>3</sup> reported that BU-based conditioning regimens were

**Table 6** Clinical outcome

	<i>ES patients (median (range))</i>	<i>Non-ES patients (median (range))</i>	<i>P-value</i>
Neutrophil engraftment (days)	10 (7–15)	10 (8–21)	0.09
Platelet engraftment (days)	13 (8–50)	12 (10–41)	0.5
Hospitalization (days)	20 (12–72)	15 (6–50)	0.001
Antibiotics (days)	10 (5–20)	7 (0–50)	0.39
Red blood cell transfusions (units)	3 (0–11)	2 (0–19)	0.01
Platelet transfusions (units)	4 (1–20)	2 (0–29)	0.01
Parenteral nutrition (days)	20 (8–65)	13 (0–35)	0.0001
Morphine (days)	7 (0–20)	0 (0–20)	0.001
Inotropics (days)	5 (0–21)	0 (0–20)	0.006
Costs (euros)	9207 (2595–52 090)	7238 (2878–32 783)	0.06

**Table 7** Causes of death (*n* = 29)

	<i>ES patients</i>	<i>Non-ES patients</i>
Infection	1	3
Multiorgan failure	2	1
Relapse	7	15

associated with ES. However, non-BU-based regimens were associated with ES in our study. This discrepancy may be due to the fact that we used non-BU-based conditioning regimens in patients with less previous chemotherapy. The association between less immunosuppression and higher risk of developing ES was already reported by Moreb *et al*.<sup>13</sup> G-CSF postinfusion is another factor invoked in the development of ES. Lee *et al*<sup>5</sup> reported that G-CSF post transplant increased the incidence of the syndrome (79% with G-CSF vs 48% without G-CSF). We did not find any influence of the use of G-CSF post transplant on ES. However, the results cannot be strictly compared because the stem cell source is different in these two studies. Ravoet *et al*<sup>3</sup> did not find an association of ES with post transplant use of G-CSF, possibly because of the small number of patients not supported by G-CSF. All patients who presented with ES in the Ravoet report received G-CSF after transplantation.

The cellular and molecular bases of ES are not completely understood. The analyses of target organs for ES have shown that different hematopoietic cells are involved in this process. For instance, in the study by Lee *et al*,<sup>5</sup> the skin biopsies showed perivascular infiltrates of CD3+ and CD4+ lymphocytes, similar to acute GVHD. Neutrophils must have a role in the development of ES because neutrophil engraftment closely precedes symptoms. Neutrophils are recruited toward sites of tissue injury and subsequently die by apoptosis. They must be phagocytosed by macrophages in order to repair the injury. It has been shown that lung inflammation is not resolved in cases of defective phagocytosis of apoptotic cells.<sup>14,15</sup> It may be hypothesized that the inflammatory lung process in the ES may be related to insufficient numbers of macrophages in the presence of high numbers of neutrophils. Supporting this is the finding that fast neutrophil recovery is associated with ES.<sup>3</sup>

Lee *et al*<sup>5</sup> described the beneficial effects of steroid therapy at standard doses, derived from its immunosuppressive effect and its anti-inflammatory nature. Maiolino

*et al*<sup>16</sup> also used steroid therapy at standard doses as treatment of ES, but they did not find significant differences on the outcome. Capizzi *et al*<sup>9</sup> used steroids at high doses, methylprednisolone at 1–2 g/day, in patients who developed perengraftment respiratory distress syndrome, and observed clinical improvement. We decided to start treatment with corticosteroids at high doses (500 mg/m<sup>2</sup>/day), tapering off as soon as possible. This resulted in a trend toward a better clinical and economic outcome. We found a trend toward higher costs in the group of patients who developed ES because more supportive care was required in these patients.

In conclusion, our data show that a high number of CD34+ cells/kg, the type of tumor, conditioning regimen and the phase of the disease are the most important risk factors for ES in children undergoing PBCT. In addition, treatment results suggest a favorable impact of early start of high doses of steroids on the outcome of ES.

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