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Peripheral blood stem cells

Engraftment syndrome in children undergoing autologous peripheral blood progenitor cell transplantation

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Summary:

There is limited experience on engraftment syndrome (ES) in children. The present study analyzes the characteristics of ES in pediatric patients undergoing autologous peripheral blood progenitor cells transplantation (PBPCT). From 1993 to 2001, 30 of 156 patients (19.2%) who underwent PBPCT developed ES (skin rash which involved more than 27% of the body surface and temperature >38.3°C with no compatible infectious disease etiology, during neutrophil recovery). Of the 30 patients who developed ES, 20 (66%) developed hypoxia and/or pulmonary infiltrates, seven (23%) had hepatic dysfunction, six (20%) developed renal insufficiency, 16 (53%) showed weight gain and three (10%) experienced transient encephalopathy. Multivariate analysis showed that the only positive predictive factor for developing ES was mobilization with high-dose G-CSF (12 μg/kg twice daily) (RR 3.88, CI 95% 1.73–8.67; $P < 0.0005$). The overall transplant-related mortality (TRM) was 8.33% and this was significantly higher in the patients who developed ES than in those who did not (23% *vs* 4.76%; $P < 0.0001$). We also found a higher morbidity in patients who developed ES, expressed as a statistically significant increase in supportive care (transfusion requirement, parenteral nutrition) and increase in the length of hospital stay. In summary, we have found ES to be the most important cause of morbidity and mortality in children undergoing autologous PBPCT.
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High-dose chemotherapy with autologous peripheral blood progenitor cell transplantation (PBPCT) is increasingly utilized in the treatment of hematologic malignancies and solid tumors.[1–3]

For years the early complications most frequently observed in patients undergoing autologous transplantation have been bacterial, viral and fungal infections, hepatic veno-occlusive disease, pulmonary complications and renal failure.

More recently, and coinciding with the increased use of peripheral blood as a source of stem cells, a potentially lethal syndrome occurring during neutrophil recovery has been described in an increasing number of patients.[4] This syndrome, known as the engraftment syndrome (ES), although differently defined by several authors, includes fever in absence of infection, skin rash and pulmonary injury which occur during early neutrophil recovery.[4–6] Furthermore, diarrhea, weight gain and capillary leak syndrome occurring at the same time have also been related to the syndrome.[7]

Although the etiology has as yet to be determined, the interaction between cytokines released during neutrophil recovery and capillary injury appears to play an important role.[8–10]

The information currently available on this syndrome in children is scanty. The present single center study analyzed the characteristics of ES in our patients.

Patients and methods

Study population

From December 1993 to June 2001, 156 pediatric patients with hematologic malignancies and solid tumors underwent autologous PBPCT at Niño Jesús Hospital. The main characteristics of the patients are shown in Table 1. Parents' informed consent was obtained in all cases.

Transplant procedure

Until 1998, PBPC were mobilized with granulocyte colony-stimulating factor (G-CSF) alone (Neupogen; Amgen, Thousand Oaks, CA, USA) once a day, at a dose of 12 μ/kg/day, subcutaneously (s.c.) for 4 consecutive days before starting apheresis.[11] During 1998 we used G-CSF at the same dose with granulocyte–macrophage colony-stimulating factor (GM-CSF, Leucomax; Novartis Pharma, Basel Switzerland) s.c. at 5 μg/kg/day.[12] From 1999 up to the present time we have used G-CSF at a dose of 12 μg/kg twice daily s.c. for 4 consecutive days before starting apheresis.[13]

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Table 1 Patient characteristics

Age (years) median (range)	8 (1–18)
Weight (kg) median (range)	26 (9–90)
Gender	
Male	99
Female	57
Diagnosis	
ALL	20
AML	19
HD	6
HNL	13
CNST	28
NB	22
PNET	5
ES	18
RB	14
WT	5
Disease status at transplantation	
1st CR	75
2nd CR	28
>2 CR	4
PR	33
PD	16
Conditioning	
Cy-TBI[a]	12
Bu-Cy[b]	42
Bu-Mel[c]	62
Bu-Th[d]	16
Other	24
Number of apheresis, median (range)	1 (1–3)
$CD34^+$ cells/kg infused, median (range)	4.03 (0.17–44.4)

Diagnosis: CNST = central nervous system tumor; NB = neuroblastoma; PNET: = peripheral neuroectodermal tumor; ES = Ewing's sarcoma; RB: = Rabdomyosarcoma; WT = Wilm's tumor.
Disease status: PR = partial remission; PD = progressive disease.
Conditioning: [a]TBI for a total dose 12 Gy and cyclophosphamide 60 mg/kg/day for 2 days; [b]busulphan for a total dose of 16 mg/kg and cyclophosphamide 60 mg/kg/day for 2 days; [c]busulphan for a total dose of 16 mg/kg and melphalan 140 mg/m^2 on day −2; [d]busulphan for a total dose of 16 mg/kg and thiotepa 250 mg/m^2/day for 3 days.

PBPC collection by large-volume leukapheresis (LVL) were performed on day +5 after mobilization by a Cobe Spectra cell separator (Cobe BCT, Lakewood, CO, USA). Details of apheresis procedures have been previously reported.[14,15]

Each apheresis product was analyzed for $CD34^+$ cell content assessed by flow cytometry using an Epics Elite flow cytometer (Coulter, Hialeah, FL, USA). The final product containing 10% DMSO was frozen using a computer-controlled freezer and stored in liquid nitrogen at −196°C.

Conditioning regimen and supportive therapy

Patients received myeloablative regimens (Table 2). All patients had a central venous line and were nursed under strict protective isolation in barrier nursing units with HEPA-filtered air. Infection prophylaxis was provided using cotrimoxazole. On day 0 collected cells were infused after rapid thawing at 37°C. One hundred and three patients received G-CSF (10 μg/kg/day) starting on day +1 until an absolute neutrophil count (ANC) $>1.0 \times 10^9$/l was maintained for 2 consecutive days, and the remaining 53 patients did not. Empiric i.v. antibiotic treatment was initiated as soon as fever of >38°C occurred. Amphotericin (1 mg/kg/day) was added if fever and neutropenia continued for 4–5 days after antibiotics were started. Blood products were infused to maintain the hematocrit >25% and platelet count $>20 \times 10^9$/l. All blood-derived transfusions were irradiated prior to use.

Table 2 Pre-transplant clinical characteristics of patients with ES (ES+) *vs* patients without ES (ES−)

	ES+ patients n = 30	*ES− patients n = 126*	*P value*
Age, median (range)	10 (1–17)	8 (1–18)	0.07
Weight, median (range)	35 (9–77)	30 (11–90)	0.1
Gender			
Male	19	80	0.8
Female	11	46	
Diagnosis			
Solid tumor	24	52	0.0002
Hematological malignancies	6	74	
Disease status at transplantation			
First remission	17	86	
Second remission	8	28	0.3
Partial remission or relapse	5	11	
Conditioning			
TBI-based	1	11	0.3
Bu-based	28	112	
Mobilization			
G-CSF 24 μg/kg/day	7	37	
G-CSF 12 μg/kg/day	18	76	0.001
G-CSF + GM-CSF	5	12	
$CD34^+$ cells infused			
$>5 \times 10^6$/kg	13	53	0.9
$>5 \times 10^6$/kg	7	72	
G-CSF post-transplantation			
Yes	15	88	0.052
No	15	37	

Definitions

Neutrophil recovery was defined as the number of days taken to achieve an ANC $>0.5 \times 10^9$/l for 3 consecutive days. Platelet recovery was defined as the time taken to achieve $>20 \times 10^9$/l without requiring transfusion. Hospital stay was defined as the number of days from day 0 to day of hospital discharge.

Engraftment syndrome (ES) was defined by skin rash clinically similar to the acute GVHD of allogeneic BMT which involved more than 27% of the body surface, and temperature of >38.3°C with no compatible infectious disease etiology associated with the early phase of hematological recovery, as reported by Lee *et al*.[16]

Transplant-related mortality (TRM) was defined as any cause of death occurring during the first 100 days post-transplant other than relapse or progressive disease.

Statistical analysis

Data are expressed as median and range. Statistical significance was determined using the Student's test when samples were normally distributed, and a non-parametric test (Mann–Whitney–Wilcoxon) when samples were not normally distributed. Results were considered significant if the P value was <0.05. Patient demographics, disease and

transplantation-related variables were analyzed for association with ES by univariate and multivariate analysis using a logistic regression model. Results are presented as relative risk (RR) and 95% confidence intervals (CI). For multivariate analysis, parameters with a probability higher than 0.1 were excluded from the regression analysis.

Results

Of the 156 patients who underwent transplantation, 30 (19.23%) met the diagnostic criteria for ES. In our study, the median of onset of ES was day +9 (range 4–19).

Of the 30 patients who developed ES, 20 (66%) developed hypoxia (SaO_2 <90%) and/or pulmonary infiltrates; seven of these 20 patients (14%) developed acute respiratory distress syndrome (ARDS) requiring mechanical ventilation. Seven of 30 patients (23%) developed hepatic dysfunction with a total bilirubin of 2 mg. Six of 30 patients (20%) developed renal insufficiency (serum creatinine twice baseline). Sixteen patients (53%) showed a weight gain >25% of baseline body weight. Three patients (10%) experienced transient encephalopathy unexplainable by other causes.

Table 2 shows the pre-transplant characteristics of the 30 patients who developed ES compared with those of the 126 patients who did not. No differences between the two groups were found for age, weight, sex, status of the disease, number of $CD34^+$ cells/kg infused or administration of G-CSF post-transplant.

Univariate analysis showed ES to be more frequent in patients with solid *vs* hematologic malignancies ($P < 0.0002$) and in those in whom mobilization was with G-CSF 12 μg/kg twice daily ($P < 0.001$).

Multivariate analysis showed that the only predictive factor for developing ES was mobilization with high-dose G-CSF (RR 3.88, 95% C.I. 1.73–8.67; $P < 0.0005$).

The clinical post-transplant characteristics of the patients with ES and those without are compared in Table 3. There were no significant differences in days to ANC $>0.5 \times 10^9$/l or to a platelet count $>50 \times 10^9$/l, but hospital stay, transfusion requirements, days on parenteral nutrition were significantly greater for the patients who developed ES.

The overall transplant-related mortality (TRM) was 8.33% and was significantly higher in the patients that developed ES than in those who did not (23% *vs* 4.76%, respectively; $P < 0.0001$).

The causes of death in the group which developed ES were ARDS (five patients) and multiorgan failure (two patients). In the group of patients who did not develop ES, the causes of death were infection (three patients), multiorgan failure (two patients) and veno-occlusive disease (one patient).

Table 3 Post-transplant clinical values of patients with ES (ES+) and patients without ES (ES−)

	ES+ patients n = 30	*ES− patients n = 126*	*P value*
Days to			
Neutrophils $>0.5 \times 10^9$/l	10 (8–14)	10 (8–12)	0.6
Platelets $>50 \times 10^9$/l	40 (11–270)	33 (11–249)	0.6
Days of i.v. antibiotics	11 (2–24)	9 (4–20)	0.04
Days of red blood cells	4 (1–19)	2 (0–13)	0.0005
Days of platelets	7 (1–38)	4 (0–29)	0.00081
Days on parenteral nutrition	24 (0–41)	15 (0–30)	0.0001
Hospital days	25 (12–84)	18 (10–29)	0.0001
TRM	23%	4.76%	0.0001

Discussion

Over the past few years PBPCT has become an alternative to bone marrow transplantation (BMT) following high-dose chemoradiotherapy in the treatment of hematologic malignancies and solid tumors in pediatric patients.[1–3,17] Coinciding with the wider use of peripheral blood as a source of progenitor cells, ES is beginning to be described more frequently.[4–6,16] The incidence of the syndrome in the adult population has been reported to range from 7 to 56%, mainly due to the use of more or less restrictive diagnostic criteria.[4–6]

Criteria for the pediatric population, however, are not available and we have used the criteria first described by Lee *et al*.[16] According to these, our incidence is 19%, although this incidence may be overestimated due to the criteria utilized. More recently, Spitzer[7] has proposed more restrictive criteria. According to these criteria, our incidence would be 12.8%, which is similar to the incidence reported by some authors in adults.[4,6] However, we cannot compare our results with other series since the present study, to our knowledge, is the first reported exclusively in children.

Like other authors, we believe that although fever and skin rash are an important part of this syndrome, the pulmonary symptoms and signs should be considered the cardinal features in the development and course of ES as is emphasized in a recent study that describes the syndrome as 'peri-engraftment respiratory distress syndrome'.[18]

Different pre-transplant factors have been related to ES. On univariate analysis we found ES to be significantly more frequent in solid tumors than in hematologic malignancies, which is similar to that of the adult series of patients with breast cancer.[5] The foregoing might be explained by the presence of a greater number of autoreactive T lymphocytes, probably involved in ES, as a result of the less intensive prior immunosuppression therapy for solid tumors than is used in hematologic malignancies.[5,19,20] Busulphan-based conditioning regimens have been described previously as related to ES development;[6] however since only 12 patients received a non-busulphan-based conditioning in this series, this association could not be studied.

Furthermore, we found that the use of G-CSF 12 μ/kg/12 h for mobilization was the most significant factor for development of ES on both univariate and multivariate analysis, a finding that has not been previously reported. The foregoing could not be explained in our series by a greater number of $CD34^+$ cells being infused, as other authors have reported in adults.[4,6] We therefore believe that this could be related to the different subpopulations of the

inoculum obtained with mobilization with high-dose G-CSF.[21] By contrast, we found no correlation with sex, conditioning regimen utilized or the administration of G-CSF post-transplant, unlike the series on adults.[4–6,16]

We have analyzed the impact of developing ES post-transplant and found a higher morbidity expressed as a statistically significant increase in supportive care (transfusion required, parenteral nutrition) and increase in the length of hospital stay.

The mortality rate from this syndrome in our series was 23%, which falls within the 18–60% range reported for adults.[4,7,22,23]

In our series, ES was found to be an important cause of mortality in children undergoing autologous PBPCT. Overall, of the 13 patients who died, seven (53%) had developed ES. This finding emphasizes the need to use standardized criteria for precise diagnosis of ES and to determine therapeutic strategies.

In adults the good response achieved with steroids in some cases of ES and diffuse alveolar hemorrhage due to their immunosuppressor or anti-inflammatory effects[17,23,24] indicates that this therapeutic strategy may be useful in children.

In summary, we have found ES to be the most important cause of morbidity and mortality in children undergoing PBPCT. It is necessary to develop standardized and specific criteria for the precise diagnosis of ES and to investigate the possible beneficial effects of steroid therapy.

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