Post-transplant events

Combined administration of alpha-erythropoietin and filgrastim can improve the outcome and cost balance of autologous stem cell transplantation in patients with lymphoproliferative disorders

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Summary:

We compared the use of G-CSF plus EPO in a group of 32 multiple myeloma and lymphoma patients with historical controls receiving G-CSF alone. Haemopoietic reconstitution was significantly faster in patients receiving G-CSF + EPO (group B), with a median time of 10 days to achieve an ANC count $> 0.5 \times 10^9$/l, compared to 11 days in the historical group (A). The median duration of severe neutropenia (ANC count < 100/ml) was significantly shorter in group B compared to group A; platelet counts $> 20 \times 10^9$ and $> 50 \times 10^9$/l were achieved at days $+13$ and $+17$, respectively in group B, compared to days $+14$ and $+24$, respectively, in group A ($P = 0.015$, 0.002) patients. The transfusion requirement was reduced in group B, with 0 (0–6) RBC units and 1 (0–5) platelet unit transfused in group B vs 2 RBC (0–9) and 2 platelet units (0–8) in group A. Median days of fever, antibiotic therapy and hospital stay were reduced in group B (9.5 days vs 22). The mean cost of autotransplantation per group A patient was 23 988 Euro, compared with 18 394 Euro for a group B patient. Our study suggests that the EPO + G-CSF combination not only accelerates engraftment kinetics, but can also improve the clinical course of ASCT.

Autologous stem cell transplantation (ASCT) has completely replaced ABMT due to accelerated engraftment kinetics and reduction of costs of the high-dose therapy (HDT).[1]

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The use of growth factors (GFs) like G-CSF or GM-CSF for blood progenitor cell (BPC) mobilization (with or without chemotherapy) allows the collection of large amounts of CD34 + cells in about 85% of patients with lymphoma or MM. The most used GF both for BPC mobilization and after transplantation is the human G-CSF (filgrastim or lenograstim) at doses ranging from 5 μg/kg, after chemotherapy, to 10–16 μg/kg as a single mobilizing agent.[2–5] Although BPCs accelerate engraftment kinetics compared to bone marrow (BM), the utility of adding GF after autotransplantation is still debated.[5–10]

Some reports concerning the timing of G-CSF administration after BPC reinfusion suggest no clear clinical benefit of early vs delayed administration.[11,12]

Consequently, in many centers the administration of G-CSF after transplantation is not performed routinely when the stem cell source is mobilized BPC, especially when the dose of CD34 + cells reinfused is above the minimum safe threshold required for a rapid engraftment.

While the BPC dose needed to ensure quick, sustained and complete engraftment should not be less than 2×10^6/kg CD34 + cells,[3,13] there is consensus that reinfusing a higher threshold (at least 5×10^6/kg) of CD34 + cells as an optimal target to warrant a safe transplant procedure in at least more than 90% of patients minimizes the risks of prolonged cytopenia and the costs of prolonged hospitalization and/or supportive care.[14,15]

Therefore, when the CD34 + cell dose is optimal ($> 5 \times 10^6$/kg), the aplastic phase after HDT is unlikely to be further reduced. Moreover, the use of larger amounts of BPC is questionable and may increase the risks of reinfusing more clonogenic tumor cells.[16,17]

The possibility of further reducing the aplastic phase after HDT has been explored by using ex vivo expansion of haemopoietic stem cells, but this approach is time-consuming and very expensive.

The ability of erythropoietin (EPO), associated with G-CSF, to reduce life-threatening neutropenia after chemotherapy, has been recently suggested by a randomized study of 50 patients with ovarian cancer,[18] and before 1995 at least three randomized studies evaluated the role of EPO administration after ASCT, but none was able to

demonstrate accelerated reticulocyte recovery or a decrease in red blood cell (RBC) transfusions.[19]

Altogether, attempts to further shorten or definitively abrogate the aplastic phase after HDT were unable to demonstrate additional significant benefits either in clinical outcome and in cost–benefit. We therefore commenced a prospective pilot study to evaluate the early combined administration of EPO and G-CSF in patients with lymphoma or myeloma after BPC reinfusion to further reduce the aplastic phase and the need for supportive care after HDT.

Patients and methods

To reduce the aplastic phase and transfusional support after HDT, we tested the combined administration of alpha-EPO + filgrastim (G-CSF) in a group of patients with lymphoproliferative disorders (lymphoma or MM), auto-transplanted with BPC and high-dose regimens including high-dose melphalan (HDM).

From December 2001, we consecutively enrolled 32 patients with MM or lymphoma, who were candidates for HDT after standard mobilization with chemotherapy followed by G-CSF. The median age was 56 years; 24 were males and eight females; 14 had MM, 17 non-Hodgkin's lymphoma (NHL) and one Hodgkin's disease (HD); five

patients with MM and two with NHL received a double autotransplant using the same regimen; consequently, the total number of HDT procedures evaluated was 39.

To identify a control group, we reviewed the medical records of all adults with MM and lymphoma autotrans-planted in our centre between January 1999 and November 2001. This control group of 33 patients (group A) was matched with the prospective group (group B) for the main clinical characteristics, including the regimen and post-transplant patient care; those patients had received only G-CSF (starting from day +5) without EPO, after HDT (Table 1).

Eligibility criteria for transplantation were the same for all patients and were based on the evaluation of performance status, the accurate evaluation of organ reserve and, in those aged over 60 years, a multidimensional geriatric assessment.[20]

The main protocol end points of the early combined administration of filgrastim plus EPO were:

(1) duration of the aplastic phase after HDT;
(2) the transfusion requirements, days of fever and days on antibiotic therapy.

The secondary end points were to compare the in-hospital days and the costs of the first 30 days after HDT in the two groups.

Table 1 Characteristics of patients

	Prospective group (B) (day +1: G-CSF+EPO)	Control group (A) (day +5: G-CSF)	P
Transplant procedures	39	40	—
Number of patients	32	33	
Sex			
Male	24	18	0.1
Female	8	15	
Diagnosis (transplant procedures)			
Non-Hodgkin lymphoma	17 (19 procedures)	18	
Hodgkin disease	1	3	0.4
Multiple myeloma	14 (19 procedures)	12 (19 procedures)	
Age			
Median	56	63	0.24
Range	23–74	20–71	
CD 34+ cells × 10^6/kg reinfused			
Median	7	5.9	0.27
Range	3.4–28.6	3.8–20.4	
Conditioning regimen			
BEAM[a]	20 procedures	21 procedures	0.82
Melphalan 200 mg/m²	19 procedures	19 procedures	
Pretransplant			
Performance Status (WHO)			
0	34 procedures	29 procedures	
1	3 procedures	9 procedures	0.6
2	2 procedures	2 procedures	
Previous chemotherapy regimens			
Median	2	3	0.36
Range	2–5	2–6	

[a]BCNU 300 mg/mq day −6, etoposyde 200 mg/mq and cytarabine 400 mg/mq from day −5 to −2, melphalan 140 mg/mq day −1.

BPC mobilization, collection and cryopreservation

All patients were mobilized with chemotherapy followed by G-CSF 5 μg/kg/day until the last leukapheresis; 14 patients with MM received cyclophosphamide 7 g/m^2; the 18 patients with NHL and HD received DHAP chemotherapy (cisplatin 100 mg/m^2 on day 1, cytarabine 2 g/m^2 on days 2–3, dexamethasone 40 mg on days 1–4).

The median number of CD34+ cells collected was 7 $\times$ 10^6/kg (3.4–28.6) with a median number of two leukaphereses; BPC were cryopreserved using an uncontrolled-rate freezing (URF) method as previously published.[21]

The patients of the control group received the same kind of mobilization according to the diagnosis and the BPC cryopreservation was also performed using the URF method.

HDT, PBSC reinfusion and patient care

All 32 patients received HDT regimens including HDM; in all cases melphalan administration was preceded by Amifostine 750 mg/m^2 intravenously (i.v.) to reduce non-haematologic toxicity as previously reported.[22] As seven patients received a double autotransplantation, the total number of HDT procedures was 39; the 14 patients with MM received only melphalan 200 mg/m^2 i.v. on day -1 for a total of 19 procedures; the 17 patients with NHL and the patient with HD received the BEAM regimen for a total of 20 procedures; the BEAM schedule consisted in the i.v. administration of BCNU 300 mg/m^2 on day -6; ARA-C 400 mg/m^2 and etoposide 200 mg/m^2 on days -5, -4, -3, -2 and melphalan 140 mg/m^2 on day -1.

On day 0, 24 h after the melphalan administration, cryopreserved BPC were reinfused after thawing in a waterbath at 37°C, using a central venous catheter.

All patients received G-CSF 5 μg/kg/day subcutaneously (s.c.) from day $+1$ until the day after the achievement of ANC $> 1 \times 10^9$/l, combined with alpha-EPO 10 000 U/day s.c., from day $+1$ for 3 weeks.

After BPC reinfusion, patients received mouth care with Clorhexidine and Fungilyn until PMN recovery; fluid administration and symptomatic therapy were administered at the discretion of the physician, but total parenteral nutrition was never administered; the antimicrobial prophylaxis and febrile episode treatment were performed as described previously.[22]

Irradiated blood products were infused to maintain haemoglobin and platelet levels above 8 g/dl and 10 $\times$ 10^9/l, respectively.

Discharge was planned when the following clinical criteria were satisfied: the patient is able to take oral therapy and to perform activities of daily living; absence of fever and no need of transfusional support for at least two consecutive days; no need of parenteral fluid administration and an available caregiver.

Patients from the control group (group A) received amifostine before HDM according to the same schedule used in patients of group B; also, the antimicrobial prophylaxis and the clinical and nursing management were performed using the same policy used in patients of group B; in particular, the criteria for starting antibiotic therapy, transfusional support, fluid administration and discharge in group A did not differ from that in group B.

Evaluation of engraftment kinetics and statistical analysis

All patients were monitored daily for the main clinical parameters and blood count to evaluate the duration of neutropenia (ANC $< 0.1 \times 10^9$ and 0.5 $\times$ 10^9/l) and the platelet and neutrophil engraftment kinetics. Neutrophil engraftment was defined as the first day on which the absolute neutrophil count (ANC) exceeded 0.5 $\times$ 10^9/l for two consecutive days. Platelet engraftment was defined as the first day on which the platelet count exceeded 20 $\times$ 10^9, and 50 $\times$ 10^9/l, unsupported by transfusion. Neutrophil nadir was defined as the first day on which ANC $< 0.5 \times 10^9$/l for two consecutive days.

We conducted a matched pair analysis comparing the engraftment kinetics and the clinical outcome in the 39 HDT procedures performed using the combination EPO + G-CSF, with the 40 HDT procedures in the control group. The two series were homogeneous both for the clinical characteristics and for the number of CD34 + cells reinfused, as verified by using the χ^2, Fisher's exact tests for discrete variables and the Mann–Whitney test for continuous variables.

We evaluated the dose of CD34 + cells reinfused, patient age, the HDT regimen, underlying disease, the number of previous chemotherapy regimens and the schedule of GF administration by the univariate and multivariate analyses of factors influencing neutrophils and platelet engraftment and duration of neutropenia. Probabilities of achieving ANC $> 0.5 \times 10^9$/l, platelet count $> 20 \times 10^9$/l and 50 $\times$ 10^9/l in the two groups (A and B) were calculated using the Kaplan–Meier method and compared by the log-rank test. Factors significantly affecting haemopoietic recovery with the univariate analysis were then included in the multivariate analysis using the Cox logistic regression model. Factors affecting the duration of neutropenia (ANC $< 0.1 \times 10^9$/l) were also analysed with the ANOVA univariate test and those found to be significant were afterwards evaluated in a multivariate linear regression model.

We also compared the clinical course of transplantation between the two groups evaluating: days of fever $> 38^\circ$C, days on intravenous antibiotic therapy, days of hospitalization from BPC reinfusion, number of RBCs and platelet units transfused during the first 30 days after transplant and the analytical costs of HDT treatment until day $+30$. The univariate ANOVA test was performed to compare transfusion requirements, duration of fever and days on antibiotic therapy; Fisher's exact test was performed to evaluate the incidence of infectious episodes in the two groups. Data were analysed using statistical software (SPSS, Chicago, IL, USA).

Cost analysis

The cost analysis was performed evaluating both the indirect cost of hospital stay not directly attributable to the treatment (overall daily room cost) and the direct costs;

the costs not directly attributable to the treatment, but not involving patient care (work time lost by patient, caregiver and productivity losses due to morbidity) were not considered.

The overall daily room cost in the Transplant Unit and the direct costs were estimated by the Health Management Office of Ancona University (directed by Professor GM Raggetti) in collaboration with the Cost Data Management of the Azienda Ospedaliera Umberto I Ancona; the costs of resources employed and the professional fees were derived from our hospital analytic accounting system; the drug costs were obtained from wholesale price lists applied to the Azienda Ospedaliera Umberto I Ancona Pharmacy (as the cost analysis was a secondary end point of this work, we omitted the detailed procedures of the economic evaluation: manuscript in preparation).

The direct costs were subdivided into four main categories:

(1) Drugs: all drugs used during the transplant procedure were considered, including conditioning, Amifostine, G-CSF, EPO, blood transfusions, antibiotics, antiemetics, fluids etc.;
(2) equipment and supplies;
(3) staff (medical and nursing);
(4) diagnostics: imaging and laboratory investigations.

In summary, the overall estimated costs of the transplant procedure in the two groups of patients included all the hospital overheads, medical and nursing staff and the room cost calculated from the day of admission in hospital until the day $+30$ after BPC reinfusion (including the costs due to a second readmission in the Transplant Unit).

Results

All 65 patients of the two groups, receiving the 79 HDT procedures, had complete and sustained engraftment. No toxic deaths were observed during the first 30 days after autotransplantation and nonhaematologic toxicity was absent or mild, consisting mainly of mucositis and diarrhoea (only 12.5% of patients had grade 3–4 WHO degree of toxicity) or neutropenic fever (in 33% of patients).

No relevant (grade 3–4 WHO) cardiac, hepatic, neurological, metabolic or renal toxicities were observed and only one patient (in group A) died within day $+90$ of sepsis, with an overall transplant-related mortality (TRM) of 1.27%; in summary, we did not observe any substantial differences between the two groups concerning major nonhaematologic toxicities.

Overall, haemopoietic reconstitution in the 79 procedures was characterized by 10 (4–25) days to achieve ANC $>0.5 \times 10^9/l$, 13 days (7–30) for platelet count $>20 \times 10^9/l$, 19 days (11–60) to achieve PLT $>50 \times 10^9/l$ and 25 days (14–190) for PLT $>150 \times 10^9/l$.

Patients needed a median of 1 RBC unit (0–8) and 1 platelet unit (0–9); the median duration of fever $>38°C$ was only 1 day (0–9) and the median number of days on antibiotic therapy was 0 (0–17).

The median duration of in-hospital stay was 18 days (4–23) overall, and 12 days from day 0 (2–28).

Comparison of the engraftment kinetics and clinical outcome in the two groups

Haemopoietic reconstitution (Table 2) was significantly faster in patients receiving the combination of G-CSF + EPO (group B), with 10 days to achieve ANC $>0.5 \times 10^9/l$, compared to 11 days in group A; the platelet count $>20 \times 10^9/l$, $>50 \times 10^9/l$ and $>150 \times 10^9/l$ (in the absence of platelet transfusions) were achieved in a median of $+13$, $+17$ and $+23$ days, respectively, in group B, compared with $+14$, $+24$ and $+50$ days, respectively, in group A patients.

Moreover, the early combination of G-CSF + EPO significantly increased the percentage of patients who quickly achieved safety levels of platelets and neutrophils: in group B, 90% of patients achieved ANC $>0.5 \times 10^9/l$ at day $+11$, platelet count $>20 \times 10^9/l$ (without transfusions) at day $+14$ and platelet count $>50 \times 10^9/l$ at day $+22$, while 90% of patients in group A achieved ANC $>0.5 \times 10^9/l$ at day $+14$, platelet count $>20 \times 10^9/l$ at day $+25$ and platelet count $>50 \times 10^9/l$ at day $+37$ (Figures 1 and 2).

Finally, the median duration of neutropenia was significantly shorter in group B, with only 3 (range 0–6) days on ANC $<0.1 \times 10^9/l$ and 5 (range 1–9) days on ANC $<0.5 \times 10^9/l$ compared to 5 (range 1–22) days and 7 (range 3–23) days, respectively, observed in group A.

As the acceleration of neutrophil engraftment in group B consisted of only 24 h (median), and even though this

Table 2 Engraftment kinetics in the two groups of patients

	Group B (day +1: G-CSF+EPO)	Group A (day +5: G-CSF)	P
Transplant procedures	39	40	—
Days to ANC $>0.5 \times 10^9/l$			
Median[a]	10	11	0.0009
CI (95%)	10–10	10–12	
Days to platelets $>20 \times 10^9/l$			
Median[a]	13	14	0.015
CI (95%)	12–14	12–16	
Days to platelets $>50 \times 10^9/l$			
Median[a]	17	24	0.002
CI (95%)	15–19	7–41	
Days to platelets $>150 \times 10^9/l$			
Median[a]	23	50	0.05
CI (95%)	16–30	1–99	
Days on ANC $<0.1 \times 10^9/l$			
Median	3	5	<0.0001
Range	0–6	1–22	
Days on ANC $<0.5 \times 10^9/l$			
Median	5	7	0.001
Range	1–9	3–23	

[a]Kaplan–Meier median.
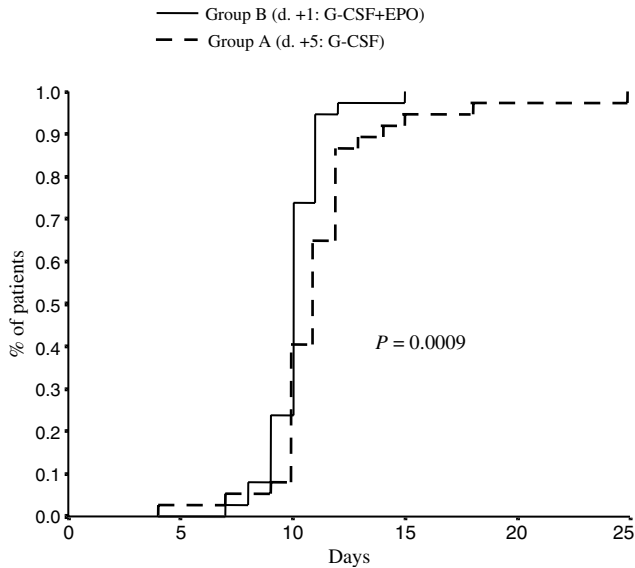
Figure 1 Probability of achieving ANC $>0.5 \times 10^9$/l in the two groups of patients.

Figure 2 Probability of achieving platelet count $>50 \times 10 \times 10^9$/l in the two groups of patients.
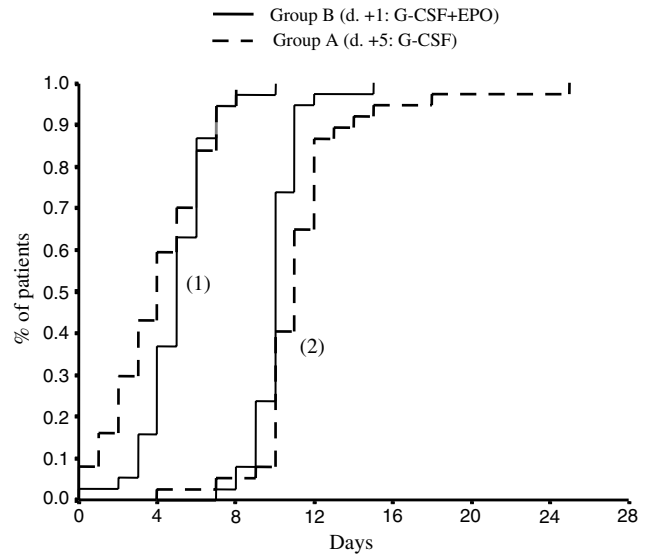

Figure 3 Kinetics of neutropenia in the two groups of patients: probability of neutrophil nadir (ANC $<0.5 \times 10^9$/l) (1) and to achieve ANC $< 0.5 \times 10^9$/l (2) after HDT.
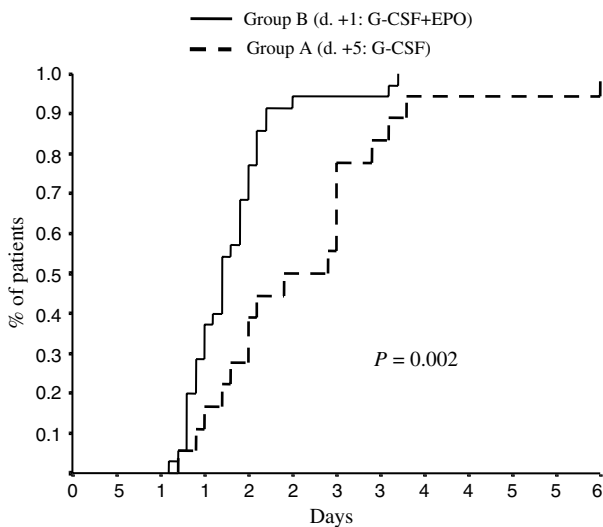
Table 3 Comparison of clinical course and supportive care in the two groups of patients

	Group B (day +1: G-CSF+EPO)	Group A (day +5: G-CSF)	P
Units of RBCs transfused			
Median	0	2	0.001
Range	0–6	0–8	
Units of platelets transfused			
Median	1	2	0.0001
Range	0–5	0–9	
Incidence of febrile episodes			
No. (%)	13/38 (34.2)	26/39 (66.7)	0.006
Febrile days ($>38°C$)			
Median	0	1	0.01
Range	0–8	0–9	
Days on antibiotic therapy			
Median	0	1	0.01
Range	0–8	0–9	

difference was statistically significant, this could not completely explain the substantial difference between the duration of neutropenia in the two groups; therefore, the combination of two factors probably contributed to significantly shorten the neutropenia in patients of group B: the acceleration of neutrophil recovery and the delay of neutrophil nadir (days to ANC $<0.5 \times 10^9$/l) after HDT, even though this delay was not statistically significant when considered alone (Figure 3).

Finally, the clinical outcome (Table 3) was significantly better in patients receiving the combination EPO plus G-CSF both in terms of days of fever and days on antibiotic therapy; patients receiving the combination EPO + G-CSF experienced a median of 0 days of febrile neutropenia (0–8) and the median number of days of i.v. antibiotic therapy was

0 (0–8). Moreover, the transfusion requirement was almost abolished in group B, with 0 RBC units transfused (0–6) *vs* 2 (range 0–8) in group A, and only 1 platelet unit transfused in group B (range 0–5) *vs* 2 (range 0–9) in group A.

Consequently, this led to a significant reduction in days of hospitalization with a median of 9.5 days (4–27), compared with 22 (15–43) in the control group of patients receiving only G-CSF.

The univariate analysis performed in the 79 HDT procedures identified the combination of G-CSF + EPO as the only significant factor ($P<0.0001$) influencing the duration of neutropenia, while CD34 + cell dose, age, type of HDT regimen, diagnosis and number of previous chemotherapy regimens were not statistically significant (data not shown).

The multivariate analysis (Table 4) confirmed this finding with a median duration of severe neutropenia (ANC $<0.1 \times 10^9$/l) of 3 days (CI 2.8–3.8) in group B, compared to 5 days (CI 4.6–7.1) in group A (CR 2.53; CI 1.7–3.9; $P<0.0001$).

The multivariate analysis selected two parameters able to predict a faster engraftment: the combination of G-CSF + EPO and the type of HDT, while all other factors were not significant; the hazard ratio for a faster achievement of ANC $>0.5 \times 10^9$/l and a platelet count $>20 \times 10^9$/l, in patients receiving the combination G-CSF + EPO, were, respectively, 1.8 and 1.72.

Also, the type of HDT significantly influenced only the PMN engraftment kinetics, but not the platelet engraftment; lymphoma patients receiving the BEAM regimen achieved the ANC $>0.5 \times 10^9$/l significantly faster (median: 10 days; 95% CI: 10–10) than those (with MM) receiving Melphalan 200 mg/m^2 (median: 11 days; 95% CI: 10–12) ($P=0.0003$). As previously shown, there were no

statistical differences concerning the diagnosis distribution and the type of HDT between the two groups of patients, and the median number of CD34 + cells $\times 10^6$/kg reinfused was not statistically different between patients with lymphoma and those with MM (data not shown).

Cost–benefit analysis

The mean estimated cost of the entire autotransplantation procedure in patients of group A was 23 988 Euro; the mean cost of the same procedure in patients of group B was 18 394 Euro.

As the costs of the transplant procedure were calculated starting from the day of the hospital admission until day + 30 post reinfusion, the additional costs due to subsequent hospitalizations needed after discharge (within day + 30) were also considered.

Table 5 shows the costs distribution in the two groups according to the categories analysed (direct costs or indirect

Table 4 Multivariate analysis of factors influencing the neutrophils and platelets engraftment and the duration of neutropenia

	Relative hazard (days to ANC $>0.5 \times 10^9$/l)	P	Relative hazard (days to platelets $>20 \times 10^9$/l)	P	Regression coefficient (days on ANC $<0.1 \times 10^9$/l)	P
Group A (G-CSF)	1	0.01	1	0.03		
Group B (G-CSF + EPO)	1.8 (95% CI: 1.14–2.96)		1.72 (95% CI: 1.1–2.8)		−2.53 (95% CI: −1.17 to −3.9)	<0.0001
Conditioning regimen						
HDM[a]	1					
BEAM	1.62 (95% CI: 1.1–2.6)	0.05	b	—	b	—
CD34 + cell $\times 10^6$/kg <5 vs >5; <7 vs $\geqslant 7$	b	NS	b	NS		
Age <60 years vs >60 years	b	NS	b	NS		
Diagnosis	b	NS	b			
Lymphoma vs Myeloma		NS		NS		
Previous chemotherapy <2 vs >2	b	NS	b	NS		

[a]High-dose melphalan (200 mg/m^2).
[b]The variable did not enter the multivariate analysis.

Table 5 Analysis of the cost of BPC transplantation procedure in the two group of patients

	Group A (day +5: G-CSF)	Group B (day +1: G-CSF + EPO)
Direct costs	*12 948 Euro*	*10 714 Euro*
Drugs (total)	3173	3914
G-CSF	440 (median 8 days)	720 (median 13 days)
EPO	—	1424 (median 20 days)
Conditioning[a]	885	885
PLT transfusions	938 (median 2 units)	469 (median 1 unit)
RBC transfusions	310 (median 2 units)	—
Others (antibiotics, antiemetics)	600	416
Equipment/materials	1656	1152
Staff (medical and nursing)	6348	4416
Diagnostic (laboratory and radiology)	1771	1232
Indirect costs	*11 040 Euro*	*7680 Euro*
Room daily cost	480 Euro	480 Euro
Days of hospitalisation[b]	23	16 days
Total	23 988 Euro	18 394 Euro

[a]Mean cost of HDT with HDM alone or BEAM.
[b]Total median number of days in-hospital until day + 30 (including readmissions).

costs) during the different phases of the transplant procedure; the cost saving in patients receiving the combination of EPO + G-CSF was achieved not only by reducing the hospital stay (mean indirect costs reduction = 3360 Euro), but also by reducing the direct costs (mean direct costs saving = 2234 Euro).

Discussion

In the last 10 years, the extensive use of mobilized BPC and the improvement of the supportive care have strongly reduced the mortality and the morbidity of ASCT, even though a small but not negligible TRM (around 2–3%) is still reported in patients with solid tumours.[23]

The growing number of HDT procedures in Western countries generated several problems related to the cost–benefit balance and quality of life of candidates for this procedure; some experience supports the use of outpatient HDT even though the careful selection of patients fit for this approach is mandatory; nevertheless, in this setting as well, the duration of severe neutropenia (ANC $<0.5 \times 10^9$/l) is still consistent (approximately 7 days).

In this setting, an original approach has been proposed by Morabito *et al*,[24] the so-called mixed Inpatient–Outpatient Model; this approach has been tested in 44 patients with MM, conditioned with HDM (and receiving EPO after BPC reinfusion) and the authors showed, in a historical comparison of 35 patients with MM, receiving the same treatment in hospital, a significant improvement of the quality of life without increasing the morbidity and mortality in the outpatient setting.

Our experience suggests that the early combined administration of EPO + G-CSF not only accelerates engraftment and reduces the duration of neutropenia, but also significantly improves clinical outcome after HDT. This translated into significant cost reduction and in the future could make outpatient transplant programs feasible and cost-effective for the majority of patients with MM and NHL, who usually receive regimens including HDM.

The role of G-CSF after ASCT has been previously investigated by several authors; however, it is still unclear if the acceleration of neutrophil recovery translates into clinical and economical benefits.[11,12,25]

In the study published by Cortellazzo,[26] a homogeneous population of lymphoma patients receiving the same kind of conditioning and reinfused with an optimal CD34 + cell dose was randomized to receive G-CSF *vs* placebo from day + 1 to stable engraftment: while only a trend in favour of G-CSF group was observed for the engraftment kinetics, no clinical benefit was observed in terms of days of hospitalization, days with fever, days on antibiotic therapy or transfusion requirement.

Recently, Hornedo[23] reported a randomised trial of 216 patients receiving HDT for breast cancer and receiving G-CSF from the day of infusion or 5 days later; patients receiving G-CSF from the day of reinfusion had faster neutrophil engraftment, but this did not translate into a reduction of hospitalization time. The authors' conclusions are that the gold standard after HDT remains late G-CSF administration, at least in patients with breast cancer.

Some *in vivo* data suggest synergy in the combination of G-CSF plus EPO, both for BPC mobilization,[27,28] and for treatment of myelodysplastic syndromes.[29] More recently, the study of Pierelli,[18] demonstrated that this cytokine combination could also be synergistic in accelerating PMN recovery after nonmyeloablative chemotherapy. Overall, these data strongly suggest a clinically relevant multilineage effect of this cytokine combination.

On the other hand, the role of EPO after autologous stem cell transplantation has been investigated in at least four randomized trials, but only two evaluated the combination of EPO + G-CSF;[19] Link reported the larger experience on 114 patients randomized to receive EPO *vs* placebo starting the day of transplantation until RBC recovery, but patients of the two groups did not receive G-CSF; furthermore, the study end points were the reticulocyte recovery and the time to RBC transfusion independence, but not the duration of the aplastic phase.[30–32]

Chao randomized a small group of 35 patients to receive G-CSF alone *vs* the combination with EPO, but in this protocol EPO was started 4 weeks before transplant and resumed on day + 1 after marrow infusion; no benefit in terms of RBC transfusion requirement was observed in patients receiving this cytokine combination, but only patients receiving bone marrow entered in this study.[33]

Vannucchi[34] compared three small groups of patients randomized to receive after transplantation the combination EPO + G-CSF, only G-CSF or neither of these cytokines; patients receiving EPO + G-CSF and G-CSF had significantly fewer units transfused than controls while the platelet and myeloid recovery were comparable in the two groups receiving G-CSF alone or combined with EPO. Besides the small number of patients enrolled (10 for each group), this study shows other important limits: all patients received BM instead of BPC (like the previous studies) and the kind of conditioning was not homogeneous in the three groups (six of 10 patients given EPO + G-CSF received TBI *vs* zero of 10 in the control group).

A summary of the main experience with G-CSF after autotransplantation (with different timing of administration) with or without EPO is reported in Table 6.

In our study, 79 consecutive ASCT procedures were performed in two groups of patients with lymphoproliferative disorders, receiving the same HDT regimen (HDM 200 mg/m² in myeloma patients and BEAM in lymphoma patients).

The two groups of patients were matched for all clinical characteristics, the CD34 + cell dose and for their management during HDT procedure: the criteria for transfusional support, antimicrobial prophylaxis and treatment of febrile neutropenia.

The main modification in our policy for lymphoma and myeloma patients receiving HDT since 1999 to 2002 was the introduction of the association G-CSF + EPO after BPC reinfusion, although we cannot exclude the possibility that some minor changes in the discharge policy may occur. In our opinion, the learning curve effect in the ASCT setting induced several temporal changes, especially during the first 10 years (1988–1998) after the introduction of GFs, which seem less important afterwards (indeed we did not observe a further improvement of TRM which is still

Table 6 Main clinical trials evaluating the role of G-CSF and EPO after ASCT

Reference	Kind of study	Kind of cytokine	PTS treated (no/diagnosis/SC source)	Engraft kinetics	Comment
Miller[19]	Randomized	Arm A: EPO (200 U/kg/d) Arm B: placebo	50 (hematol. malignancies/BM)	No statistical difference	No improvement in RBC transfusion requirement
Khwaja[12]	Retrospective	Group A: G-CSF Historical group: no GFs	17 (malignant lymphomas/BM)	Significantly improved in group A	Cost saving with delayed G-CSF
Link[32]	Randomized	Arm A: EPO (150 U/kg/d) Arm B: placebo	114 (hematol. malignancies/BPC)	No statistical difference	No improvement of red blood cells requirement
Chao[33]	Randomized	Arm A: Epo (600 U/kg × 3/w)+G-CSF Arm B: placebo+G-CSF	35 (lymphoma/BM)	No statistical difference	No improvement in red blood cells or platelets requirement
Spitzer[6]	Randomized	Arm A:G-CFS (7.5 µg/kg/d) and GM-CSF (2.5 µg/kg/d) Arm B: no GFs	37 (miscellaneous/BM and BPC)	Significantly improved in the group receiving GFs	Shorter hospitalization in the GFs group; no difference in clinical outcome and transfusion requirement
Shimazaki[10]	Prospettico	G-CSF (50 µg/m²) Vs no GFs	20 (hematol. malignancies/BPC)	Significantly improved in the group receiving G-CSF	Reduced antibiotic use in the GFs group
Vey[25]	Retrospective	Group A: G-CSF from d +1 Group B: G-CSF from d +6 Group C: no GFs	78 (NHL,HD,breast cancer,ovarian cancer/BM)	Significantly improved in the groups A and B; no difference between A and B	No difference in clinical outcome and cost saving between the two groups
Cortellazzo[26]	Randomized	Group A: G-CSF Group B: no G-CSF	40 (NHL/BPC)	No statistical difference	No difference in clinical outcome or platelet transfusion requirement
Klumpp[8]	Randomized	G-CSF Vs no GFs	41 (miscellaneous/BPC with or without BM)	Significantly improved in the group receiving G-CSF	Shorter hospitalization and significant reduction of antibiotics in the GFs group
Vannucchi[34]	Randomized	Arm A: G-CSFfrom d+1 Arm B: G-CSF plus EPO (150 U/kg) from d+1 Arm C: no GFs	30 (HD,NHL,ALL/BM)	No statistical difference between group receiving G-CSF and group receivimg G-CSF+EPO; significantly improved reticulocyte kinetics in arm B	No transfusional benefits
Hornedo[23]	Randomized	Arm A: G-CSF from d 0 Arm B: G-CSF from d+5 Arm C: no GFs	241 (breast cancer/BPC)	Significantly improved in the two arms receiving G-CSF; no difference between arm A and arm B	Shorter hospitalization in arms A and B; no difference between A and B; arm C closed due to delayed engraftment

2–3%). Our data are derived from a matched-historical comparison and should be confirmed in a randomized study or by increasing the number of patients autotransplanted in the two groups in a prospective fashion.

Multivariate analysis confirmed that, in this homogeneous setting of patients receiving an optimal dose of CD34+ cells, the only two factors able to significantly accelerate engraftment and reduce the aplastic phase were the EPO + G-CSF combination and the BEAM regimen. We can exclude that the type of conditioning could be responsible for the different engraftment kinetics between the two groups: indeed there was no difference in the distribution of diagnosis between the two groups and finally BEAM only influenced the kinetics of ANC engraftment and not platelet engraftment.The clinical benefits of the early combination of EPO + G-CSF mainly consisted in the abolition of RBC transfusions, in the reduction of febrile neutropenia with reduced parenteral antibiotic need and, most importantly, these observations suggest a substantial improvement in the safety of the HDT procedure.

Indeed, 90% of patients in group B achieved ANC $> 0.5 \times 10^9/l$ at day +11 with only 3.3 days of severe neutropenia, compared to 5.8 days of severe neutropenia in patients of group A who achieved ANC $> 0.5 \times 10^9/l$ (in 90% of cases) at day +14.

The reduction of the aplastic phase was the main end point of our study and these results strongly suggest a synergistic effect of EPO + G-CSF, even though a larger number of patients should be treated with this combination to confirm these data; as well, the consequent reduction of days of hospitalization that we observed need to be prospectively confirmed in a multicentre experience. It should be stressed that the reduction of days of hospitalization in group B was not a cosmetic effect, but this advantage was maintained even considering the additional days of re-hospitalization during the first 30 days after ASCT (16 vs 23 days).

In the Freeman study,[1] a detailed cost analysis was conducted in a large number of patients receiving autotransplantation for NHL from 1989 until 1995. Many factors contributed to a significant cost decrease: the shift from BM harvest to BPC mobilization, the use of different kinds of HDT regimens (including or not TBI in many patients) and the use of different techniques of BPC collection. The main cost saving was achieved after 1992, and this probably reflects improvements in ABMT technology and patient care; also, the duration of hospital stay decreased from a mean of 42.5 to 11.9 days by 1995, but it was not specified if the duration of hospitalization included also the conditioning or it was computed by day 0.

In our experience, the mean cost saving for each transplant procedure by using the combination of G-CSF + EPO was 24% despite the more extensive use of expensive drugs such as EPO in group B, which were largely counterbalanced by reductions in hospitalization, transfusional support and the working time of the medical and nursing staff.

Recently, Reiffers[35] reported preliminary data in 27 patients with MM, suggesting the possibility of abrogating postmyeloablative chemotherapy neutropenia by *ex vivo* expansion of autologous CD34+ cells; all patients experienced an impressive reduction of neutropenia with only 2 days of severe neutropenia (ANC $< 0.5 \times 10^9/l$) and only 1 day of platelets $< 20 \times 10^9/l$; the transfusion requirement was also reduced but not abrogated, with a median of 1 (0–9) platelet transfusions and 0 (0–3) of RBC transfusions.

Bertolini *et al*[36] previously published a limited experience of megakaryocytic precursor generation in an 'ex vivo' system, to use for platelet support during the aplasia post-auto-PBSC transplantation: 10 cancer patients received escalating doses of autologous megakaryocytic progenitors generated by *ex vivo* liquid culture, but only two of the 10 patients receiving the expanded cells did not need platelet transfusions. More recently, Blair *et al*[37] evaluated the 'ex vivo' expansion of megakaryocyte progenitors and obtained promising results. Unfortunately, all these approaches are time-consuming, very expensive and require sophisticated devices for large-scale CD34+ cell selection, GMP-approved systems for cell expansion in bags and at least 1 week of 'ex vivo' culture before clinical use.

Our approach seems feasible in the majority of patients who are candidates for HDT, and in centres without facilities for the conventional outpatient management of patients.

Until now, we have only preliminary data concerning the impact of this combination on the quality of life, but our experience suggests that this simple approach could effectively make the outpatient ASCT a feasible and cost-effective procedure in a large number of patients. Indeed, we have initiated up a prospective trial to evaluate this approach for the outpatient management of ASCT.

Acknowledgements

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