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Review

Individual Quality Assessment of Autografting by Probability Estimation for Clinical Endpoints: A Prospective Validation Study from the European Group for Blood and Marrow Transplantation

Francesco Lanza [1,2,*], Diana C. Campioni [2], Andrzej Hellmann [3], Giuseppe Milone [4], Anders Wahlin [5], Jan Walewski [6], Pierangelo Spedini [1], Cristina Fiamenghi [1], Antonio Cuneo [2], Wanda Knopińska [3], Monica Swierkowska-Czeneszew [6], Jordi Petriz [7], Stefan Fruehauf [8], Dominique Farge [9], Mohamad Mohty [10], Jacob Passweg [11], Tapani Ruuto [12], Alejandro Madrigal [13], Hans E. Johnsen [14] for the Quality Assessment of Haematopoietic Stem Cell Grafting Committee of European Blood and Marrow Transplantation Society

[1] *Hematology Institute, Hospital of Cremona, Italy*
[2] *St. Anna University Hospital, Hematology Section, Ferrara, Italy*
[3] *Hematology Service, Medical Academy of Gdansk, Poland*
[4] *Section of Hematology, Ospedale Ferrarotto BMT Unit, Catania, Italy*
[5] *Umea University Hospital, Sweden*
[6] *Maria Sklodowska-Curie Memorial Institute and Oncology Centre, Warsaw, Poland*
[7] *Hospital Vall d'Hebron, Barcelona, Spain*
[8] *Zentrum für Tumordiagnostik und therapie Osnabrück, Germany*
[9] *Department of Internal Medicine, Paris, France*
[10] *Department of Hematology, Nantes, France*
[11] *Hematology Service, Basel, Switzerland*
[12] *BMT Unit, Vienna, Austria*
[13] *Antony Nolan Foundation, London*
[14] *Aalborg University Hospital, Aalborg, Denmark*

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A B S T R A C T

The aim of supportive autografting is to reduce the side effects from stem cell transplantation and avoid procedure-related health disadvantages for patients at the lowest possible cost and resource expenditure. Economic evaluation of health care is becoming increasingly important. We report clinical and laboratory data collected from 397 consecutive adult patients (173 non-Hodgkin lymphoma, 30 Hodgkin lymphoma, 160 multiple myeloma, 7 autoimmune diseases, and 28 acute leukemia) who underwent their first autologous peripheral blood stem cell transplantation (PBSCT). We considered primary endpoints evaluating health economic efficacy (eg, antibiotic administration, transfusion of blood components, and time in hospital), secondary endpoints evaluating toxicity (in accordance with Common Toxicity Criteria), and tertiary endpoints evaluating safety (ie, the risk of regimen-related death or disease progression within the first year after PBSCT). A time-dependent grading of efficacy is proposed with day 21 for multiple myeloma and day 25 for the other disease categories (depending on the length of the conditioning regimen) as the acceptable maximum time in hospital, which together with antibiotics, antifungal, or transfusion therapy delineates four groups: favorable ($\leq$7 days on antibiotics and no transfusions; $\leq$21 [25] days in hospital), intermediate (from 7 to 10 days on antibiotics and $<$3 transfusions, $\leq$21 to 25 days in hospital or $\geq$7 days on antibiotics and no transfusions; from 21 to 30 days [25 to 34] in hospital), unfavorable ($>$7 days on antibiotics, $>$3 but $<$6 transfusions; $>$30/34 days in hospital after transplantation), and very unfavorable ($>$10 days on antibiotics, $>$6 transfusions; $>$30 to 34 days in hospital). The multivariate analysis showed that (1) PBSC harvests of $\geq 4 \times 10^6$/kg CD34 + cells in 1 apheresis procedure were associated with a favorable outcome in all patient categories except acute myelogenous leukemia and acute lymphoblastic leukemia ($P = .001$), (2) $\geq 5 \times 10^6$/kg CD34 + cells infused predicted better transplantation outcome in all patient categories ($P < .0001$) except acute myelogenous leukemia and acute lymphoblastic leukemia, (3) 1 or 2 aphereses ($P = .001$) predicted good outcome, (4) toxicity increased with higher graft volume reinfused ($>$500 mL) ($P = .002$), and (5) patients with a central venous catheter during both collection and infusion of PBSC had a more favorable outcome post-PBSCT than peripheral access ($P = .007$). The type of mobilization regimen did not affect the

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* Correspondence and reprint requests: Francesco Lanza, MD, Section of Hematology & BMT Unit, Hospital of Cremona, Via Concordia N. 1, 26100 Cremona, Italy.
E-mail address: f.lanza@ospedale.cremona.it (F. Lanza).

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outcome of auto-PBSCT. The present study identified predictive variables, which may be useful in future individual pretransplantation probability evaluations with the goal to improve supportive care.

INTRODUCTION

Enumeration of CD34+ cells was shown to be useful in the procedure of stem cell mobilization and harvest from blood for transplantation, and it seems to be informative for the prediction of fast or delayed 3-lineage engraftment and blood cell recovery after high-dose therapy [1–8]. In 1998 the European Joint Accreditation Committee of the International Society of Hematotherapy and Graft Engineering/European Group for Blood and Marrow Transplantation (EBMT) prepared a regulatory document on the standards for blood and marrow progenitor cell collection, processing, and transplantation [9]. The major objectives were to promote quality of medical and laboratory practice in hematopoietic progenitor cell transplantation [9]. These standards extend and detail pre-existing international activity to ensure that appropriate standards of work and product quality are established and maintained [3,4,6,9].

The aim of supportive autografting is to reduce the side effects from high-dose therapy and avoid procedure-related health disadvantages for patients at the lowest possible cost and resource expenditure. Economic evaluation of health care is becoming increasingly important. Clinical endpoints for such evaluations have been proposed [1,2] in which the first objective was to analyze efficacy, which in the context of post-transplantation supportive care may be defined by, for example, days on antibiotics and transfusions of blood products; the second objective was to analyze toxicity defined by time to blood cell recovery; and the third objective was to analyze safety as defined by risk of early relapse or death. Based on such retrospective analyses, we estimated prognostic models regarding the short-term graded endpoints in a heterogeneous group of patients in a prospective registration and validation study [1,2,10–13].

In this study, we report data collected prospectively from 397 consecutive patients who underwent their first autologous peripheral blood stem cell transplantation (PBSCT) based on a myeloablative conditioning regimen. We considered primary endpoints evaluating efficacy (ie, health economic issues, including antibiotic administration, transfusion of blood components, and time in hospital), secondary endpoints evaluating toxicity (in accordance with Common Toxicity Criteria [14]), and tertiary endpoints evaluating safety (ie, the risk of regimen-related death or disease progression within the first 12 months after PBSCT) [15].

METHODS

Objectives and Endpoints

Based on the proposal from the EBMT subcommittee on quality assessment of autologous stem cell graft, pretransplantation variables were analyzed for influence on aggregated clinical endpoints chosen as indicators for the outcome of post-transplantation supportive care, toxicity, and safety as defined in Table 1. The first objective was to analyze cost/expenditure of supportive care by an aggregate endpoint termed efficacy, defined by days on antibiotics and use of transfusions of blood products. The second objective was to analyze the reduction of marrow toxicity from high-dose therapy by an endpoint termed toxicity defined by the time to blood cell recovery. The third objective was to describe patient outcome by an endpoint termed safety defined by early death or disease recurrence within 12 months. The endpoints were binary graded as favorable or unfavorable, as defined in Table 1. The selection of 21 and 25 days as the optimal hospitalization time was due to the length of the conditioning regimen (1 day for multiple myeloma [MM] receiving high-dose melphalan and 6 days for Hodgkin lymphoma [HL] and non-Hodgkin lymphoma [NHL] receiving the BEAM regimen).

Table 1
Proposed Graded Clinical Endpoints in Quality Assessment Based on a 4-Stage Grading Classification to Evaluate the Health Economic Efficacy

Objective	Endpoint	Grading
Primary: health economic efficacy (to study the efficacy of stem cell graft reinfusion)	Days on antibiotics, transfusion of blood components, days in hospital after transplantation	*Favorable:* ≤7 days on antibiotics and no transfusions; ≤21 days in hospital for MM and ≤25 for other disease categories (NHL, HL, AML, acute lymphoblastic leukemia, autoimmune disease) *Intermediate:* from 7 to 10 days on antibiotics and <3 transfusions; ≤21 days in hospital (<25 for other disease categories), ≥7 days on antibiotics and no transfusions; 21< × <30 days in hospital *Unfavorable:* >7 days on antibiotics, >3 but <6 transfusions; >30 days in hospital in MM and >34 for other disease categories *Very unfavorable:* >10 days on antibiotics, >6 transfusions; >30 days in hospital in MM and >34 in other disease categories
Secondary: toxicity (to evaluate toxicity after stem cell graft reinfusion)	Days to ANC >.5 × 10^6/L and platelets > 20 × 10^6/L Other organ toxicity if appropriate, as defined by WHO for mucositis, dermatitis, and enteritis	*Favorable:* ANC and platelet recovery before 14 days and no major organ toxicity *Unfavorable:* ANC and platelet recovery after 14 days and/or organ toxicity A toxicity novel classification proposed by combining grade, type, and number of adverse events: Low: grade 0-1 and n <2 events Intermediate: grade 1 and n >2 events High: grade 1 and n >2 or grade 2 and n <2 events Very high: grade 2 or 3 and n >2 events or grade 4 or 5
Tertiary: safety and disease progression (to evaluate safety after stem cell graft reinfusion)	Death or disease recurrence	*Favorable:* Alive and without disease progression after 12 months *Unfavorable:* Death or disease progression before 12 months

ANC indicates absolute neutrophil count; WHO, World Health Organization.

Patient Characteristics

Included in the study were 397 consecutive patients (study code Q01). This was an EBMT observational study released at the end of 2004 (December 2004), and patient accrual ended in December 2011. The following European teams provided case report forms to the secretary of the study: Hematology Section, St. Anna Hospital, Italy (CIC 330); Section of Hematology, Ospedale Ferrarotto BMT Unit, Italy (CIC 792); Umeå University Hospital, Sweden (CIC 731); Hematology Service, Medical Academy of Gdansk (CIC 799) Poland; Hematology Section, Hospital of Cremona, Italy (CIC 226); Spain Hospital de Navarra (CIC 577); Section of Hematology, University of Warsaw (CIC 800), Poland; Hospital Virgen de la Arrixaca (CIC 323), Spain; and University Hospital, Lublin (CIC 695), Poland.

Median patient age was 51 years (range, 21 to 70). One hundred and seventy three patients had NHL, 30 patients had HL, 160 patients had MM, 10 patients had autoimmune diseases (8 with systemic sclerosis and 2 with multiple sclerosis), 24 patients had acute myelogenous leukemia (AML), and 4 patients had acute lymphoblastic leukemia. Performance status, evaluated by the Eastern Cooperative Oncology Group (ECOG) system, was assigned as follows: 222 patients were assigned to ECOG 0, 101 patients ECOG 1, and 74 patients ECOG 2.

Patients were treated with different chemotherapy regimens, according to local policy. A single line of chemotherapy was administered in 58% of the cases before the mobilization regimen; 42% of patients received more than 1 line of chemotherapy (range, 2 to 4). At the time of mobilization, disease status was as follows: among NHL patients, 97.2% were in complete remission and 2.8% in partial remission. Among MM patients, 64% had achieved a very good partial remission and 30% of patients a partial remission; a complete remission was achieved in the remaining MM cases (6%). Complete remission before the stem cell mobilization procedure was also reached in patients with AML, acute lymphoblastic leukemia, and other diseases, according to EBMT criteria. Only 3% of patients received radiotherapy before mobilization.

Patients enrolled in this study were treated as follows. The 273 NHL patients (18 low-grade, 155 high-grade patients) were treated with 1 or 2 lines of chemotherapy. Most patients were treated with R-CHOP (110 patients), MACOP-B (26 patients), or CEOP (24 patients). The 29 patients treated with 2 lines of chemotherapy received R-DHAP or R-ESHAP. Ten patients affected by indolent NHL received treatment with a fludarabine-based regimens, whereas 16 received R-CHOP or R-CVP. The 30 HL patients were treated with ABVD chemotherapy regimen in 24 cases and BEACOPP in the remaining ones. Among these patients, 9 were treated with 1 line of chemotherapy, and 23 patients were treated with 2 or more lines with a range of cycles between 3 and 8. For MM, 160 patients with newly diagnosed myeloma were included in the study. Fifty-five were treated with VAD regimen (range, 4 to 6 cycles); 42 were treated with thalidomide plus dexametasone and 3 with thalidomide, melphalan, and steroids (MPT, 6 to 14 cycles), whereas the 50 remaining patients were treated with bortezomib plus dexamethasone.

Patients with acute leukemia were treated with standard chemotherapy regimens (ICE, DCE, or similar protocols), and complete remission was assessed using standard criteria. Patients with autoimmune diseases were treated according to ASTIS and ASTIMS trials.

Stem Cell Harvest

Cells were harvested when the leukocyte count in PB was at least >1000 cells/μL and the CD34+ cell count was >15/μL. The mean number of apheresis procedures was 2.43 (±.16; 95% confidence interval, 0 to 6), and 9.94 liters of blood (±.30; 95% confidence interval, .02 to 13.8 liters) were meanly processed in the single procedure.

Mobilization and Conditioning Regimen

A total of 183 patients received high-dose cyclophosphamide (≥4 g/m^2) and 31 low-dose cyclophosphamide (<4 g/m^2). Among patients with NHL, 46 received DHAP/ESHAP regimens; 26 received etoposide in combination with daunorubicin or methotrexate or carboplatin as a mobilization regimen; cytarabine (ARA-C) in combination with methotrexate or cyclophosphamide was administered in 25 patients; 18 patients received ifosfamide in combination with gemcitabine or vincristine or etoposide or ARA-C; and 43 patients received CHOP/CEOP mobilization regimens. Finally, 78 patients received only granulocyte colony-stimulating factor for PBSC mobilization.

The granulocyte colony-stimulating factors used alone or in combination with chemotherapy were as follows: lenograstim in 153 cases (1 to 2 vials per day), filgrastim in 226 patients (5 to 10 μg/kg/day); pegfilgrastim in 18 patients (13 patients at the dose of 6 mg and 5 patients at 12 mg), and plerixafor in 15 cases. Plerixafor was used in poor mobilizer patients who failed a previous attempt of mobilization [16–20].

The conditioning regimens adopted in this study for auto-SCT were as follows: BEAM (carmustine, etoposide, cytarabine, and melphalan) regimen for 152 patients affected by NHL; mitoxantrone 60 mg/m^2, plus melphalan 180 mg/m^2 for 13 patients with NHL; thiotepa-CY, Bu-Cy, or Bu-Mel for AML patients; and other regimens for other patient categories. In MM patients 148 received melphalan 200 mg/m^2, 6 patients received melphalan 100 mg/m^2, and 6 patients received a combination of melphalan and mitoxantrone. All patients received a myeloablative conditioning regimen. In 90% of the cases, granulocyte colony-stimulating factor administration started 1 to 5 days after auto-SCT and continued until the absolute neutrophil count was greater than 1000/μL for 3 consecutive days.

Venous Access

A central venous catheter was introduced in 317 patients before auto-SCT, whereas 80 had no central venous catheter, and transplantation was performed using a peripheral venous access.

Data Collection (MED C Form)

Data were collected through a specific MED C form composed of seven forms: (1) registration form (patient characteristics), (2) mobilization phase (type and dosage of cytokine used and of chemotherapy regimen), (3) graft evaluation form (type of apheresis machine; apheresis program; volume of blood processed; duration [hours + minutes]; venous access; volume collected and information on the leukocyte, hemoglobin, and platelet count; complications during the collection procedure; total CD34 number; type of CD34 analysis [single or dual platform analysis]; cryopreservation procedure [automated freezing, freezing medium, storage conditions]; reinfusion; and graft manipulation), (4) adverse events form, (5) hospitalization form, (6) supportive care form (use of antibiotics, dosage, and duration), and (7) serious adverse events form, in which we listed a great number of information relevant to the study [21,22]. Data from the MED C form were combined with data reported in MED A and, when available, with the MED B form (see: http://www.ebmt.org/).

A time-dependent grading of efficacy was proposed with day 21 in MM and 25 days for other disease categories as the acceptable maximum time in hospital, which together with antibiotics, antifungal, or transfusion therapy delineates 4 groups: an acceptable outcome for patients discharged before day 22 with no therapy, and an unacceptable outcome for patients who stay in hospital >21 days on continuous therapy [15]. A 4-stage grading classification is shown in Table 1 [1,15].

Toxicity was evaluated in accordance with Common Toxicity Criteria, as defined by the World Health Organization or derived references (ECOG, cancer research cooperative group, etc.) for mucositis, dermatitis, and enteritis as well as grading of hematological toxicity after stem cell infusion [14]. Concerning toxicity, the proposed grading system is time-independent, with outcome assessed according to the World Health Organization recommendation for grading of organ toxicity as acceptable if toxicity is grades 0 to 2 and unacceptable if grades 3 to 4. By tradition, the hematological toxicity has to be time-dependent, and an evaluation has been proposed (Table 1).

Finally, evaluation of "safety" by mortality and disease recurrence indicates an acceptable outcome if patients are alive with no disease recurrence up to day 100 and unacceptable if the high-dose therapy is followed by death or relapse before 1 year after PBSCT. Mortality and disease recurrence was also evaluated 1 year after autologous bone marrow transplantation. By combining efficacy, toxicity, and safety parameters, a novel classification is proposed as shown in Table 1.

Statistical Analysis

The following variables were analyzed as potential factors influencing post-transplantation outcome: age, pathology, bone marrow involvement at the time of mobilization, prior chemotherapy and radiation therapy, and type of mobilization regimen. The potential effect of the various factors on the response variables (ie, CD34+ cell counts measured under the different settings) were preliminarily assessed using Spearman's rank correlation coefficients, and the differences between cohorts were evaluated both by the nonparametric Kruskall-Wallis analysis of variance or by the Mann-Whitney 2-sample test and, for Gaussian variables, by parametric analysis of variance followed by LSD post-hoc comparisons.

Multivariate regression analysis was applied to determine in a stepwise fashion the relative rank of independently significant pretransplantation and transplantation-related variables. The prognostic variables were used to estimate probability for the outcome (acceptable or unacceptable), depending on the level of CD34 numbers in the harvested graft. The following prognostic models were considered in the analysis: sex, age, disease, mobilization method, type of growth factor used, graft volume, number of apheresis procedures performed, conditioning regimen, post-transplantation growth factor administration, and number of reinfused CD34+ cells. The univariate and multivariate statistical analyses were

Table 2
Mobilized Blood Stem Cells, Collection, and Auto-PBSCT

Characteristic	Median (Range or %)
CD34+ peak (day after the start of granulocyte colony-stimulating factor with/without chemotherapy)	12 (5-21)
White blood cell count in concomitance with CD34+ peak (day after the start of granulocyte colony-stimulating factor with/without chemotherapy)	3.1 (2-56)
Number of apheresis performed	2.2 (1-4)
Volume of apheresis processed	12,370 mL (7000-15,900)
Number of collected CD34+ cells $\times$ 10^6/kg	8.1 (.6-23)
Number of collected CD34+ cells $\times$ 10^6/kg per single apheresis	1.3 (.5-14)
Number of patients collecting $>2 \times 10^6$/CD34+ cells	277/397 (69%)
Number of patients harvesting a total number of CD34 $\times$ 10^6/kg/LP >1.5 but ≤ 2 CD34+ $\times$ 10^6/kg/LP	66 (16%)
Number of patients harvesting a total number of CD34+ $\times$ 10^6/kg/LP >1 but ≤ 1.5 CD34+ $\times$ 10^6/kg/LP	30 (8%)
Patients harvesting a total number of CD34+ $\times$ 10^6/kg/LP $>.5$ but ≤ 1 CD34+ $\times$ 10^6/kg/LP	24 (6%)
Volume infused of PBSC preparation at the time of transplantation, mL	373 (125-1020)
Absolute number of infused CD34+ cells ($\times 10^6$/kg)	3.9 (1.01-12.5)

conducted first in the whole patient group and second by focusing on the groups formed by NHL, MM, and AML patients [15].

RESULTS

Circulating Blood Stem Cells, Collection, and Auto-PBSCT

The main clinical and laboratory data are given in Table 2. Data are expressed as a median and range. Sixty-nine percent of patients reached a good level of progenitor cell content in the apheresis product (3.9 CD34+ $\times$ 10^6/kg/leukapheresis; range, 2 to 16.7); in particular, 136 patients harvested a total amount of CD34 $\times$ 10^6/kg/LP, >2 and ≤ 5 CD34+ $\times$ 10^6/kg/LP and 122 patients harvested a total amount of CD34+ $\times$ 10^6/kg/LP <5.

Of note, $<1\%$ (.6% of the cases) of stem cell harvests were manipulated before transplantation. Most of them performed CD34+ cell selections (CliniMacs, Miltenyi Biotec, Cologne, Germany), and in 4 patients a purging procedure was performed using maphosphamide.

After PBSCT, engraftment of neutrophil granulocytes occurred at a median day 12 (range, 9 to 25), whereas platelets engrafted at day 15 (range, 10 to 27). Patients who received manipulated grafts showed a delayed engraftment for both granulocytes and platelets (days 17 and 23, respectively). However, this patient subgroup comprised a very small number of patients and therefore did not affect the statistical significance of our data analysis.

Supportive Care

The efficacy of autografting was evaluated considering the following supportive care parameters: number of days with fever on antibiotics, number of transfusions, and hospitalization time. In 26.9% of patients examined, prophylactic antibiotics were administered, and in 61.5% of patients antibiotic therapeutic administration was used for a mean number of 16 days. The median number of days for the therapeutic use of antimicrobial agents was 11.3.

The use of prophylactic antibiotics was found to be associated with a significantly lower incidence ($P < .01$) of infectious disorders and, in general, with a more favorable outcome post-PBSCT. In 4 cases, a bacterial contamination of the apheresis product was observed; these patients were treated with a targeted use of antibacterial therapy.

As for most parameters analyzed, transfusion of red blood cells and platelets was 1.8 (range, 0 to 18) and 2.9 (range, 0 to 14), respectively. The median time of hospitalization was 26 days (range, 14 to 65). Finally, efficacy results, evaluated with our 4-stage grading classification (Table 1), were as follows: 16% of patients were included in stage 1 (favorable), 46% of patients were included in stage 2 (intermediate), 34% of patients were included in stage 3 (unfavorable), and 4% were included in stage 4 (very unfavorable) (Figure 1).

The secondary endpoint of the study was to evaluate toxicity. Results from our novel classification (Table 1), described in Methods, showed that 35.7% of patients experienced a low toxicity, 23.8% of patients an intermediate toxicity, 14.1% of patients a high toxicity, and 25.5% of patients a very high toxicity (Figure 2).

We considered the grade and the type of the adverse event, and the combination of the 2 parameters give rise to the following classification: low, toxicity grades 0 to 1 (31.2%); intermediate, toxicity grade 2 (40.9%), high, toxicity (22.2%); and very high (5.5%).

The tertiary endpoint of the study was to evaluate the safety after stem cell graft infusion considering regimen-related death or disease recurrence. Concerning severe adverse events, we found in 1.5% of patients a death event, in 1.8% of patients a severe adverse event occurred, in 2.19% of patients a new and follow-up event occurred, and 4.7% of patients (7 of 319) required rehospitalization. Disease relapse occurred in .9% of patients at day 100 and in 6.8% within 1 year after auto-PBSCT in the NHL/HL patient group. The frequency of disease relapse 1 year after auto-PBSCT was 25% in MM and 42% in acute leukemia patients. At 12 months after PBSCT, the death rate was 3.4%.

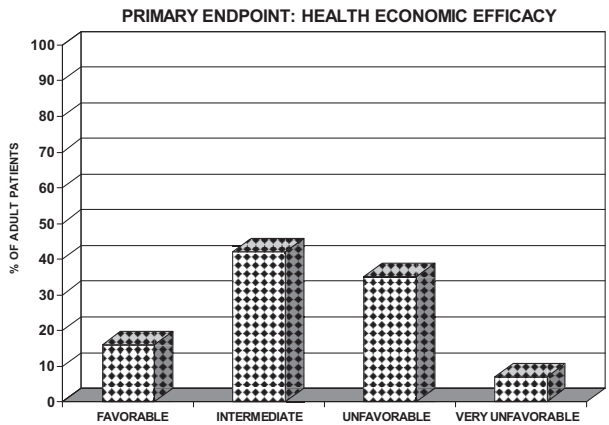

Figure 1. Distribution of patients in the various categories according to the clinical endpoint related to the health economic efficacy. Assessment of this endpoint is based on the evaluation of the following parameters: antibiotic administration, transfusion of blood components, and hospitalization time.

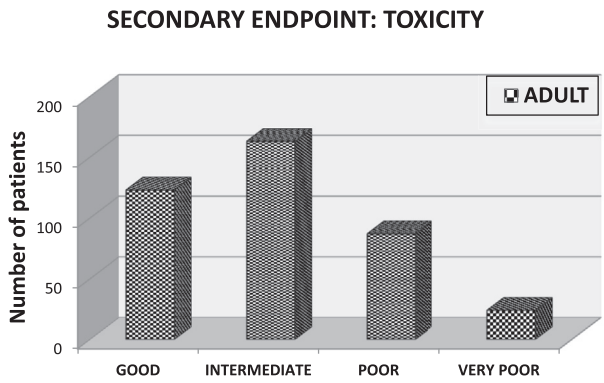

Figure 2. Distribution of patients in the various categories according to the clinical endpoint related to toxicity. Assessment of this endpoint was done in accordance with Common Toxicity Criteria. The proposed grading system is time-independent with outcome assessed according to the World Health Organization recommendation for grading of organ toxicity as acceptable if toxicity is grade 0-2 and unacceptable if grade 3-4. By tradition, hematological toxicity has to be time-dependent and an evaluation has been proposed, according to four grades: (1) low: grade 0-1 and n <2 events; (2) intermediate: grade 1 and n >2 events; (3) high: grade 1 and n >2 or grade 2 and n < 2 events; (4) very high: grade 2 or 3 and n >2 events or grade 4 or 5.

Univariate and Multivariate Analysis

In the statistical analysis, the first point of the study, efficacy, was regarded as "quality." In univariate analysis, "quality" seems to be affected by venous access; in fact, patients with a central venous catheter were associated with a significantly better outcome after PBSCT than patients with peripheral venous access ($P = .007$). Furthermore, "quality" decreases with CD34+ volume reinfused >500 mL ($P = .0001$). Finally, adverse events higher than grade 3 were found to be a statistically significant unfavorable factor ($P = .0001$).

Concerning toxicity, the number of apheresis procedures was found to be statistically significant, as 1 or 2 apheresis procedures was associated with less toxicity ($P = .001$). Toxicity increased with CD34+ cell volume reinfused >500 mL. Finally, patients classified as poor in relation to toxicity showed poor outcome post-PBSCT ($P = .0001$), according to our grading system.

In multivariate analysis, toxicity had a negative effect on transplantation outcome because patients who experienced higher toxicity had a poor quality transplantation ($P = .0001$). Finally, a higher CD34+ cell volume reinfused (>500 mL) was found to increase toxicity with a statistically significant P value ($P = .002$).

Concerning PBSC collection, a smaller number of apheresis procedures (1 or 2) was found to be statistically significant to predict good outcome compared with a larger number of apheresis procedures (3 to 6; $P = .001$). The number of CD34 cells collected in each apheresis was found to be a favorable factor, since the number of CD34+ cells collected $>4 \times 10^6$/kg in 1 apheresis predicted good outcome, except for AL patients. Finally, a number of CD34+ cells infused $> 5 \times 10^6$/kg was found to be a further favorable factor for stem cell engraftment. No correlation among the type of mobilization regimen, type of conditioning regimen, type of growth factor used for the mobilization regimen, and outcome of autologous transplantation was documented in this study. As far as the degree of toxicity and the assessment of the overall quality of transplantation is concerned, a center effect was clearly documented ($P < .01$).

DISCUSSION

Supportive reinfusion of progenitor cells after SCT ultimately aims to re-establish hematopoiesis after an initial recovery of end-stage blood circulating cells to a level necessary for reducing the risk of side effects, such as infections, bleeding, or anemia [10–13]. It is known that a graft content of more than 5 million CD34+ cells/kg body weight is safe, resulting in fast recovery of absolute neutrophil count and platelets before days 14 and 21, respectively, in a major fraction of patients and, most important, only has a minor risk for engraftment failures [10,23–33]. The aim of supportive autografting is to reduce the side effects from high-dose therapy and to avoid procedure-related health disadvantages for patients at the lowest possible cost and resource expenditure. Economic evaluation of health care is becoming increasingly important. Clinical endpoints for such evaluations have been proposed [1,2]. The first objective of this proposal was to analyze efficacy, which in the context of post-transplantation supportive care may be defined by, for example, days on antibiotics and transfusions of blood products. The second objective was to analyze toxicity defined by time to blood cell recovery. The third objective was to analyze safety as defined by risk of early relapse or death. Based on such retrospective analyses, we estimated prognostic models regarding the short-term graded endpoints in a heterogeneous group of patients in a prospective registration and validation study.

In our study, multivariate analysis showed that either the number of CD34 + cells collected and reinfused $>4 \times 10^6$ cells/kg in 1 day or the number of CD34+ cells collected and reinfused $>5 \times 10^6$ cells/kg is associated with more rapid engraftment and better transplantation outcome, except for acute leukemias patients [26–28]. The number of patients with autoimmune disease was low, so the prognostic value of CD34+ cells in this disease category still remains uncertain. Moreover, it is obvious from such data that we will never obtain an exact number of CD34+ cells delineating an "insufficient" or "safe" graft. We have to reconsider these terms and change exact numbers into probabilities of obtaining clinical efficacy, avoiding toxicity, and retaining safety, with evaluation by proper endpoints [1,2,15,34,35].

In the last decade, hundreds of reports have based their conclusions about quality assessment on surrogate markers, and, as suggested, it seems to be time for a move toward evaluation based on clinically relevant factors. Such data, although published from single centers, are not available from multicenter trials and have to be generated in a prospective manner. It is worth mentioning that the introduction of PBSCT has not changed the risk of documented infections compared with the use of conventional bone marrow grafts, although a faster neutrophil recovery is substantially documented.

Health, economic, and life-quality considerations need to be included in assessment of supportive hematopoietic cell therapy. Of less importance may be the side effects (eg, hematological toxicity) as defined by time to lineage recovery or engraftment. This protocol has proposed a change by assessing the probabilities of obtaining primary, secondary, or tertiary endpoints, therefore providing evidence that engraftment and hematopoietic recovery are not the only clinical endpoints in autografting. Such an assessment may allow us to handle each patient individually in daily practice by predicting not only the efficacy, but also the risk of side effects as well as safety.

To this aim, in this study we first evaluated the efficacy of autografting by analyzing clinical endpoints beyond the CD34+ cell number, such as supportive care, time in hospital, and so on, and proposing a time-dependent grading of efficacy, as previously reported. Our results of 397 patients who underwent an auto-SCT emphasized that only a lower number of patients were graded as good for efficacy.

In our analysis, we observed several factors affecting toxicity in PBSCT. We did not focus just on the number of CD34+ cells reinfused but considered the volume of CD34+ cells reinfused. We observed that toxicity increased with higher volume of CD34 + cells reinfused (>500 mL). Few studies investigated the occurrence and severity of adverse events after auto-SCT [21,23–30], and most of them focused on the dimethyl sulfoxide activity affecting toxicity or on the amount of leukocyte in the apheresis product. None, however, analyzed the CD34+ cell volume reinfused as we did. Another important factor to predict good outcome in this prospective study was the low number (1 or 2) of apheresis procedures compared with a higher number of apheresis procedures. This parameter is not often considered because CD34+ cells collected and reinfused are preferred. In our study, it seemed to be important to predict low toxicity and a better transplantation outcome.

Moreover, we considered as a secondary endpoint of the study hematological and extra-hematological toxicity with a time-independent grading system. As previously reported, we observed that patients with poor-grade toxicity had a worst transplantation outcome [1,2].

Today, hematological toxicity is considered one of the more important secondary endpoints for graft evaluation, which in practice focuses on the primary impact of health economic endpoints. A proposed analytic strategy of such pre-transplantation variables defines clinically relevant primary and secondary endpoints as, for example, supportive transfusions or antibiotic administration and toxicity, respectively. Introduction of such endpoints that are binary graded as acceptable or unacceptable post-transplantation outcomes may allow us to estimate prognostic models and illustrate individual quality assessment based on probability evaluation.

Taken together, these data confirm that further variables other than the CD34+ cell number play a role in transplantation outcome. These findings underscore the need for a more extensive quality assessment of autologous PBSCT, taking into account that transplantation outcome is influenced not only by graft content, but also by other important parameters such as time in hospital, supportive care, assessed in terms of blood transfusion and antibiotic therapy, and grade of toxicity, and the occurrence of relapse or death after transplantation.

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