Significance of graft quality in allogeneic hematopoietic stem cell transplantation

Semnificația calității grefei în transplantul allogenic de celule stem hematopoietice

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Abstract

Purpose of the study. *The content of the graft plays a major role for the outcome in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). This retrospective study aims to assess the correlation between the graft content and chimerism on one hand and on the other hand to find the predictive role of the graft content on engraftment kinetics.* ***Material and method.*** *Sixteen grafts were analyzed before transplantation by flowcytometry and chimerism in terms of percent of donor cells was prospectively investigated at specific time points either by real-time polymerase chain reaction (PCR) or by fluorescence in situ hybridization (FISH). Correlations between the two parameters were assessed by using SPSS 8.0 for Windows.* ***Results.*** *There was no significant correlation between the CD34+ progenitor content of the graft and percent of donor cells at the investigated time points. Instead, our data shows that the T cell content of the graft correlated significantly with the percent of donor cells on days +30 and +90. The respective Pearson coefficients were $r = 0.692$ ($p < 0.01$) on day +30 and $r = 0.575$ ($p < 0.05$) on day +90. A significant correlation was also found between the CD34+ cell content and time to engraftment ($r = - 0.767$; $p < 0.01$).* ***Discussion.*** *Chimerism is a predictive parameter used to assess post-transplant disfunctions. The significant correlations of the T cell graft content with the chimerism can further predict outcome. The higher the number of CD34+ cells in the graft, the earlier the patient engrafts.*

Key words. *Allogeneic hematopoietic stem cell transplantation, flowcytometry, chimerism, engraftment.*

Rezumat

Obiectivul studiului. *Conținutul grefonului joacă un rol important pentru evoluția pacienților supuși unui transplant alogenic de celule stem hematopoietice (TCSH). Obiectivul acestui studiu retrospectiv a fost pe*

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de o parte evaluarea corelației dintre conținutul grefonului şi chimerismul post-transplant iar pe de altă parte stabilirea rolului predictiv pe care îl exercită conținutul grefonului asupra asupra kineticii de grefare. ***Material şi metodă.*** *Au fost analizate flowcitometric şaisprezece grefoane înainte de transplant iar chimerismul din punct de vedere al procentului de celule de la donor a fost şi el analizat prospectiv prin reacția de polimerizare în lanț (real-time PCR) la momente prestabilite sau prin hibridizare fluorescentă in situ (FISH). Au fost analizate corelațiile dintre cei doi parametri utilizând ca software pentru prelucrarea statistică a datelor SPSS 8.0 for Windows.* ***Rezultate.*** *Nu a putut fi evidențiată o corelație semnificativă statistic între conținutul de celule CD34 din grefon şi procentul de celule de origine de la donor la momentele prestabilite de investigare a chimerismului. În schimb, datele noastre evidențiază o corelație semnificativă între procentul de celule stem de la donor în ziua +30 şi + 90 respectiv şi conținutul de limfocite T din grefon. Coeficienții Pearson respectivi au fost r = 0.692 (p < 0.01) în ziua +30 şi r = 0.575 (p < 0.05) în ziua +90. Conținutul in celule CD34 al grefonului s-a corelat semnificativ cu timpul de grefare (r = - 0.767; p < 0.01).* ***Discuții.*** *Chimerismul reprezintă un parametru predictiv utilizat pentru a evidenția disfuncțiile post-transplant. Corelațiile semnificative dintre conținut de limfocite T din grefon şi chimerism comportă şi ele o valoare predictivă. Cu cât numărul de celule CD34 din grefon este mai mare cu atât grefarea se produce mai precoce.*

Cuvinte cheie: *Transplant de celule stem hematopoietice alogenic, flowcitometrie, chimerism, grefare.*

Introduction

The stem cell graft, whether consisting of bone marrow or growth factor-mobilized peripheral blood mononuclear cells, contains a variety of cells which differ as to their function or their capacity of survival in the host. The most important cells in the graft though are the CD34+ progenitor cells which have the capacity of hematopoietic reconstitution after hematopoietic stem cell transplantation (HSCT). An adequate dose of CD34+ cells is necessary for autologous as well as for allogeneic peripheral blood stem cell transplants (PBSCT), in order to ensure engraftment, a low content of CD34+ cells in the graft being associated with graft failure or graft insufficiency and consequently a higher morbidity and mortality. Another important component of the graft are the lymphocyte subpopulations, especially the CD3+ T cells which proved to exert an important influence on the outcome of HSCT; they induce on one hand a negative effect by determining the graft versus host disease (GvHD) and, on the other hand, play a role in the graft versus leukemia (GvL) or graft versus malignancy (GvM) effect.

The term chimerism refers to the presence of lymphohematopoietic cells of non-host origin in the recipient (1). It has been well established that after allogeneic HSCT, a dynamic chimera is created by the presence of donor-derived cells in the recipient. This status makes it necessary to accurately assess the chimerism in the patients' blood or bone marrow in order to provide key information on the presence of engraftment, on iminent rejection or even on relapse (2). Since both chimerism status and graft quality have been associated with the clinical outcome of patients after allogeneic HSCT, the aim of the present study was to determine the correlation between the quality and cellular content of the graft and the chimerism status and implicitly the association with the post-HSCT clinical outcome.

Material and methods

This is a retrospective study conducted on a lot of sixteen patients with allogeneic HSCT from matched related donors (MRD) performed in the Center for Bone Marrow Transplantation Timisoara between 2003 and 2008. In all donor-recipient pairs, HLA histocompatibility was 100%. Patients' characteristics are shown in *Table 1*.

Table 1. Patients characteristics

Parameter	Number of patients/ transplants (percentage)
Number of patients	16
Age of patients	
< 5 years	2
5 – 10 years	6
10 – 15 years	1
> 15 years	7
Donor/patient sex mismatch	6 (37.5%)
Underlying disease	
Acute lymphoblastic leukemia	6 (37,5%)
Acute myeloid leukemia	5 (31,25%)
Chronic myeloid leukemia	1 (6,25%)
Severe aplastic leukemia	3 (18,75%)
Chronic granulomatous disease	1 (6,25 %)
Chimerism analysis by RT-PCR	9 (56.25%)
Chimerism analysis by FISH	6 (37.5%)
Chimerism not assessed*	1 (6.25%)

** - not assessed due to death prior to day +30*

Donors and stem cell harvest protocol

Bone marrow was collected from three healthy sibling donors and administered unmanipulated to the respective recipients. In other 13 sibling donors, the stem cell harvest was performed by mononuclear cell apheresis from the peripheral blood after a prior mobilization regimen consisting of the administration of 10 µg/kg/day filgrastim (G-CSF). On the 5^{th} and 6^{th} day of mobilization, mononuclear cell apheresis was performed in order to reach a target dose of 5×10^6 CD34+ cells/ kg recipient but if this target could not be achieved, a dose of 3×10^6 CD34+ cells/ kg recipient was considered as acceptable for stable engraftment.

CD34+and CD3+ cell count in the graft

For each donor, two tubes were prepared and processed in parallel from the peripheral blood apheresis product (PBAP): (i) double-staining CD34PE/ CD45PerCP and (ii) triple-staining CD4FITC/ CD8PE/ CD3PerCP. Samples were incubated with antibodies 20 min. in the dark, at room temperature. The next step was the red cells lysis with BD Facs Lysing solution followed by centrifugation 5 min at 300g, then cells washing with cell-wash, resuspension and acquisition of 100000 events. Cells were acquired on a three-color FACS Calibur flow cytometer (Becton Dickinson Biosciences) equipped with a 488 nm laser and analyzed with CellQuest 3.1 software.

Gating strategy applied for CD34+cells detection was the two-platform ISHAGE protocol (CD34+ cells are detected based on four parameters: CD45PerCP/CD34PE/side and forward single light scatter). T-lymphocytes were determined as the percentage of cells that reacts with anti-CD3 FITC (fluorescein isothiocyanate). T-lymphocyte subpopulations expressing CD3+CD4+ and CD3+CD8+ were calculated as percentages of total lymphocytes CD3+.

Chimerism analysis

Analysis of chimerism in terms of determination of the percentage of donor cells in the recipient's peripheral blood was performed by real-time PCR in the case of donor-patient sex match and by fluorescence *in situ* hybridization (FISH) for those cases with donor-patient

Table 2. Descriptive statistics of the graft content

	CD34+ (x10^6/kg)	CD3+ (x10^8/kg)	MNC (x10^8/kg)	CD3+CD4+ (x10^8/kg)	CD3+CD8+ (x10^8/kg)
Number of patients	16	16	16	16	16
Mean	5.0667	2.6700	5.2560	1.4680	0.8160
Median	3.8700	3.2000	4.6000	1.1800	0.7400
Std. Deviation	2.6453	1.1976	2.7464	1.1954	0.6423
Minimum	2.30	0.30	0.88	0.12	0.05
Maximum	11.30	4.00	13.12	5.04	2.87

MNC = mononuclear cells contained in the graft

sex mismatch. Time-points of chimerism analysis were days: +30, +90, +180, +270, +365 post-transplant. The description of the employed methods for chimerism analysis was presented by us in another recent publication and therefore will not be detailed in this paper.

Statistical analysis

Data was analyzed by using SPSS 8.0.0 for Windows® software in order to establish correlations (Pearson coefficient) between the graft content and percentage of donor cells in the peripheral blood of the recipient at specific time-points after the HSCT. Scatter plots were drawn in order to illustrate significance of correlations. Correlation was assessed with the chimerism on time-points +30, +90 and +180 after transplantation. Day 0 was considered the day of the transplantation.

Results

Before the criopreservation procedure, the graft was analyzed in terms of the CD34+ and CD3+, CD3+CD4+ and CD3+CD8+ cell content. Descriptive statistics of the graft content are displayed in *Table 2*.

The graft content, in terms of number of CD34+ progenitor cells, varied among the different patients and there was also a difference between stem cell sources, bone marrow (BM) versus peripheral blood apheresis product (PBAP). Overall, the median number of CD34+ cells was 3.87 x 10^6/kg recipient with a minimum of 2.3 x 10^6/kg recipient obtained from BM and 11.3 x 10^6/kg recipient in a graft obtained in the PBAP. Regarding the T cell content, the grafts contained a median number of 3.2 x 10^8/kg recipient with a minimum of 0.3 x 10^8/kg recipient and a maximum of 4 x 10^8/kg recipient.

Correlations were performed by using SPSS 8.0.0 for Windows® software in order to determine whether there is a direct relationship between the graft content (expressed in absolute count of cells/kg recipient) and the global quantitative donor chimerism (expressed in % donor cells in the peripheral blood of the recipient) at the time points specified above. Since the majority of patients presented either a transient mixed chimerism or a complete donor chimerism at day +180 post-transplantation, except for 3 patients with a progressive mixed chimerism, correlations were performed only up to day +180 post-transplant. Our analysis showed that there was no significant correlation between the CD34+ content of the graft and donor chimerism at the investigated timepoints. Instead, our data shows that the T cell content of the graft correlated significantly with the global chimerism on days +30 and +90. The respective Pearson coefficients were $r = 0.692$ ($p < 0.01$) on for day +30 and $r = 0.575$ ($p < 0.05$) on day +90 respectively (*Figures 1 and 2*). There was no significant corelation between the two parameters on day +180. In order to assess the effect of the graft content on time to engraftment which, by definition, is the time from the day of transplantation (day 0) to the day when neutrophil

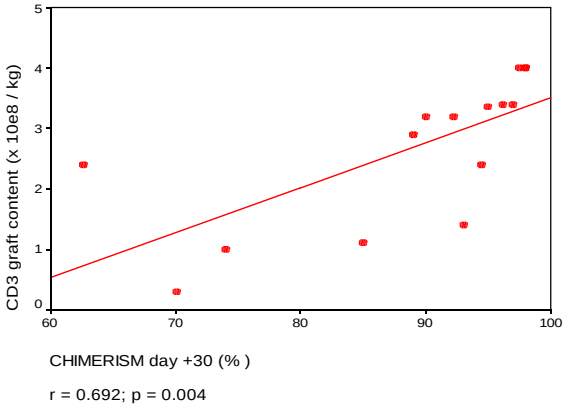

Figure 3. Correlation CD3-chimerism (day +30)

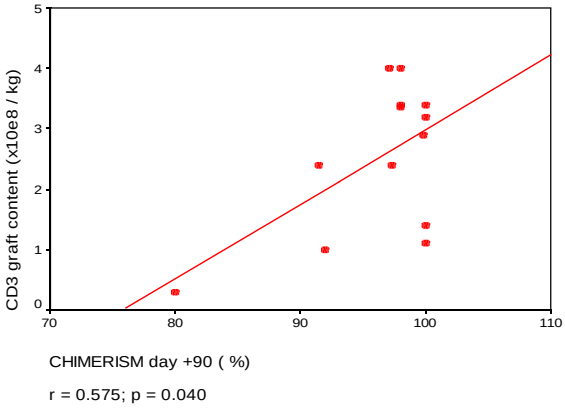

Figure 5. Correlation CD3-chimerism (day +90)

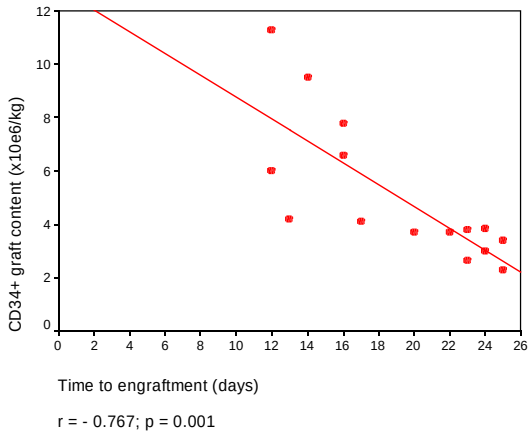

Figure 9. Correlation of CD34 cell content and engraftment

count is > 0.5 x 10^9/L in the peripheral blood, we correlated the CD34+ cell content of the graft with the days needed to reach engraftment. The correlation was significant and is illustrated in *Figure 3* ($r = -0.767$ and $p < 0.01$). Neither the CD3+ cell content nor the mononuclear cell (MNC) content did correlate with time to engraftment.

Discussion

Because chimerism has a major predictive value for the outcome of HSCT with respect to engraftment, GvHD, graft rejection or failure, we aimed at assessing the relationship between the graft content and chimerism and the possible association between graft content and engraftment time.

In order to obtain a sustained and complete engraftment in the setting of allogeneic HSCT the European Bone Marrow Transplant Registry (EBMT) recommends a minimum of 2 – 3 x 10^6 CD34+ cells/kg recipient (3). It is also widely accepted nowadays that the cell content of the graft exerts a major impact on the outcome of transplantation with respect to engraftment kinetics, development of acute and chronic GvHD, graft rejection or graft failure and implicitly chimerism (4 – 6, 8). Many studies which assessed this impact have issued a series of conclusions, especially with respect to the CD34+ cell content and the CD3+ T cell content. It has been shown that cell content differs when comparing BM with PBAP as a stem cell source, the PBAP containing up to a 5 fold higher total number of nucleated cells, 3 – 4 times more CD34+ cells and 10 – 20 times more CD3+ lymphocytes compared to BM (6). Another study published by Briones et al. showed that allogeneic PBSCT with T-cell depleted grafts lead to a significantly higher percent of complete donor chimerism in the recipients as compared with allogeneic bone marrow transplantation (BMT) using T-cell depleted allografts. The reason for this was associated with the higher content of CD34+ progenitors in the PBSC graft when

compared to BM grafts, the T-cell count after depletion being similar in the two mentioned stem cell sources used (7). This hypothesis was also supported by another study group which revealed a rate of complete chimerism of only 25% in the T-cell depleted BMT cohort as compared with 97.4% in the T-cell depleted PBSCT cohort, due to the increased amount of CD34+ progenitor cells in the latter (8).

In our study we observed that there was no significant correlation between the CD34+ progenitor cell amount in the graft and the chimerism assessed at time-points +30, +90, +180 which, of course, does not diminish the role which the amount of CD34+ progenitors from the graft plays for donor cell engraftment but it neither sustains the hypothetic predictive value of the graft CD34+ cell content for the kinetics of the chimerism after transplantation.

Major concerns have been raised regarding the relationship between the T cells in the graft and GvHD or between the low number of this lymphocyte subset in the graft and graft failure. Some studies focused on the different T lymphocyte subsets showing that the CD8+ lymphocyte subset is actually involved in the GvHD and that the depletion of these cells from the graft would preserve the GvL effect (5). The amount of CD3+ T-cells in the graft remain a leading cause for acute and chronic GvHD and it has also a direct impact on engraftment kinetics (4-6, 8). In order to improve engraftment kinetics in children receiving T-cell depleted allografts for inherited metabolic storage diseases, Gaipa et al. reintroduced a small amount of CD3+ cells back in the initially T-cell depleted graft in order to have enough T cells to favor engraftment and on the other hand being on the ”safe side” by preventing GvHD. The same procedure was employed by other centers as well, showing that T cells play a major role in influencing donor engraftment, alloreactivity of donor cells and implicitely chimerism which has proved to be highly predictive for the outcome of HSCT (9).

Since there is not sufficient data in literature regarding the direct relationship of the initial graft T-cell content at the time of transplantation and the kinetics of chimerism, our data revealed some interesting issues. *Figures 1 and 2* show that there is a significant correlation between the CD3+ cell amount and the global chimerism on day +30. There was a stronger correlation with the donor chimerism on day + 30, correlation which diminished in time, probably due to the fact that the majority of the patients reached full donor chimerism or had a transient mixed chimerism with a decreasing pattern starting with day +90 towards day +180.

Regarding the relatioship between the CD34+ progenitor cell amount in the graft and time to engraftment, our findings confirm data published in literature. The higher the number of CD34+ progenitors, the earlier did the patients engraft. *Figure 3* shows the inverted correlation between the two parameters with a negative Pearson coefficient.

Overall our data support other similar results published in literature making the graft content an important predictive tool for the outcome of patients with matched related donor allogeneic transplantation. The T cell content of the graft can predict the percentage of donor cells in the peripheral blood of recipients in the first three months after the transplantation, this way providing information regarding the potential of the graft to produce a complete donor cell engraftment. Further research is mandatory in order to find the optimal CD34+ progenitor and lymphocyte subset graft content for the major goals of transplantation: short time of engraftment, sustained engraftment, good antitumor effect but concomitant avoidance of GvHD.

Disclosure statement on conflict of interest

No actual conflict of interest exists among the authors of this study nor is there the appearance of any conflict of interest (either currently or potentially). No special funds have been raised for the preparation of this study.

Abbreviations

BM = bone marrow;
BMT = bone marrow transplantation.
FISH = fluorescence *in situ* hybridization;
FITC = fluorescein isothiocyanate;
G-CSF = granulocyte - colony stimulating factor;
GvHD = graft versus host disease;
GvL = graft versus leukemia effect;
GvM = graft versus malignancy;
HLA = human leukocyte antigen;
HSCT = hematopoietic stem cell transplantation;
MRD = matched related donor;
PBAP = peripheral blood apheresis product;
PBSCT = peripheral blood stem cell transplants;
PCR = polimerase chain reaction.

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