

CORRELATIONS BETWEEN GRAFT COMPOSITION AND IMMUNE RECONSTITUTION IN AUTOLOGOUS STEM CELL TRANSPLANTATION – A SINGLE CENTER STUDY

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SUMMARY:

Background. Hematopoietic stem-cell transplantation has evolved as the best therapeutic option for many patients with hematologic malignancy. The conditioning regimen given before transplantation eliminates virtually all pre-existing immunity. The resulting immune deficits leave the host susceptible to a variety of infections, many of which carry significant morbidity and mortality. These deficits are resolved over time with re-constitution of the immune system. **Aim:** to analyze the immune reconstitution after autologous peripheral stem cell transplant and its possible correlation with the graft composition. **Material and Method:** The retrospective observational study was conducted on 70 patients who underwent autologous hematopoietic stem cell transplant, out of which 70% with frequent relapses or resistant malignancy. The conditioning regimen was polychemotherapeutic (BEAM, BU/CY, MEL) in all cases. Median administered CD34 cell dose was $5.63 \pm 7.01 \times 10^6/\text{kg}$ while for mononuclear cells (MNC) it was $3.13 \pm 2.38 \times 10^8/\text{kg}$. The immune status was evaluated four times/year at 3 months interval by flowcytometry with a panel for CD3+, CD4+, CD8+, CD19+, CD16+, CD56+. **Results:** The most delayed reconstitution was observed for CD3+ CD4+ lymphocytes with a recovery of 35% at 1 year. At 3 years the recovery was 80%. Regarding CD19+ lymphocytes a 100% reconstitution was achieved at day +100. The recovery of CD16+CD56+ lymphocytes was 40% at 1 year post transplantation. More severe in autologous setting, this immunodeficiency did not influence in a significant modality the global rate of infection. Instead the severity of post-transplant infections was life-threatening: 2 of the patients developed severe pneumocystis pneumonia, 14 of them experienced reactivation of clinical of varicella zoster virus and 1 patient presented aspergillosis. No significant correlation was observed between the graft composition and the immune reconstitution. **Conclusions:** Post transplantation global immunodeficiency in our patients was more severe and more prolonged compared to data published in the specialized literature, probably due to the intense regimens administered prior to the autologous transplant. All patients had a low CD4+/CD8+ ratio during at least the first year post-transplant, caused by a persistent increase of CD8+ lymphocytes and a constant reduction of CD4+ lymphocytes, determining the increase to susceptibility infections for a prolonged period of time post-transplant.

Key Words: immune reconstitution, autologous peripheral stem cell transplant, graft composition

CORELAȚII ÎNTRE COMPOZIȚIA GREFONULUI ȘI RECONSTITUȚIA IMUNĂ ÎN TRANSPLANTUL AUTOLOG DE CELULE STEM HEMATOPOIETICE – STUDIU UNICENTRIC

Rezumat: Introducere: Transplantul de celule stem hematopoietice a devenit cea mai bună opțiune terapeutică pentru mulți pacienți cu hemopatii maligne. Regimul de condiționare pre-transplant distruge imunitatea pre-existentă. Deficitul imun rezultat face ca pacienții să fie susceptibili la o varietate de infecții, multe dintre acestea cu morbiditate și mortalitate crescută.

Received for publication: 12.10.2009
Revised: 19.11.2009

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Rezumat (continuare): Aceste deficiențe se pot rezolva în timp prin refacerea imunității.

Obiectiv: analiza reconstituției imune după transplantul autolog de celule stem hematopoietice și posibila corelație cu compoziția grefonului administrat.

Material și Metodă: Studiul retrospectiv observațional s-a realizat pe un lot de 70 de pacienți la care s-a efectuat transplant medular autolog de celule stem hematopoietice. 70% dintre aceștia au prezentat recăderi multiple sau boală chimiorezistentă. Regimul de condiționare a fost polichimioterapic (BEAM, BU/CY, MEL) în toate cazurile. Cantitatea medie de celule CD34+ administrate a fost de $5.63 \pm 7.01 \times 10^6/\text{kg}$ în timp ce pentru celulele mononucleare a fost de $3.13 \pm 2.38 \times 10^8/\text{kg}$. Statusul imun a fost evaluat la interval de trei luni prin metoda citometriei în flux cu un panel pentru CD3+, CD4+, CD8+, CD19+, CD16+, CD56+.

Rezultate: Cea mai întârziată reconstituție a fost observată în cazul limfocitelor CD3+CD4+, cu o recuperare de 35% la 1 an și 80% la 3 ani. În ceea ce privește limfocitele CD19+, refacerea de 100% a fost atinsă în ziua +100. Refacerea limfocitelor CD16+CD56+ a fost de 40% la 1 an post-transplant. Imunoreconstituția severă în cazul transplantului autolog nu a influențat în mod semnificativ rata globală a infecțiilor, dar severitatea acestor infecții post-transplant a fost amenințătoare de viață. 2 pacienți au prezentat pneumonie cu *Pneumocistis carinii*, 14 au prezentat reactivări clinice ale virusului varicelo-zosterian și un pacient a fost diagnosticat cu aspergiloză. Nu s-a observat corelația semnificativă între compoziția grefonului și reconstituția imună.

Concluzii: Imunodeficiența globală post-transplant a fost mai severă și mai prelungită în cazul pacienților noștri comparativ cu datele publicate în literatura de specialitate, probabil datorită tratamentului chimioterapic intensiv pre-transplant. Toți pacienții au prezentat, cel puțin în primul an post-transplant, un raport CD4+/CD8+ scăzut cauzat de creșterea persistentă a limfocitelor CD8+ și de reducerea constantă a limfocitelor CD4+, fapt care a dus la creșterea susceptibilității pentru infecții o perioadă lungă de timp post-transplant.

INTRODUCTION

Hematopoietic stem-cell transplantation (HSCT) has evolved as the best therapeutic option for many patients with hematologic malignancy. [1,2] Results of randomized trials in recent years suggest that high-dose chemotherapy followed by infusion of autologous HSCs can offer prolonged disease-free survival in hematologic malignancies including Hodgkin and non-Hodgkin's lymphoma in relapse, acute myelogenous leukemia, and multiple myeloma. [3,4] Similar results, clinically encouraging, have been obtained in the treatment of solid tumors. [5,6]

After transplantation, reconstitution of bone marrow (BM) consists in two distinct phenomena, numerical recovery of BM cellular elements on the one hand and functional recovery of cellular interactions on the other.

Although reappearance of neutrophils and platelets is often considered the endpoint of hematologic recovery after intensive chemotherapy and stem cell transplantation, this ignores the second aim of BM recovery, that of immunological reconstitution. In fact, functional recovery of lymphoid and immune effector cells occurs very gradually and reconstitution of normal humoral and cellular immunity may take more than a year. This serious aspect that affect the evolution of transplanted patients has been in our attention for years. [7,8]

In Romania, the first autologous HSCT was performed in 2001 in the Bone Marrow Transplant Unit of the

Emergency Hospital for Children, Louis Turcanu, Timisoara. The experience of our center is limited due to many hindrances and disadvantages mainly related to the late addressability of patients (after prolonged and intensive chemo- and radiotherapy regimens). This reduces the chance of adequate stem cell prelevation and thus negatively influences the quality of the graft. Another undesired consequence relates to the immune reconstitution following HSCT which is severely impaired in patients submitted to multiple regimes of chemotherapy. Given these conditions the quality and quantity of the cellular constituents of the graft have represented a priority for our team.

MATERIAL AND METHOD

Study subject

We included in the study 154 grafts, representing those used for the 70 procedures of autologous HSCT. The conditioning regimen was polychemotherapic (BEAM, BU/CY, MEL) in all cases.

Graf harvesting

Peripheral hematopoietic stem cell (PHSC) harvest has been performed in all 70 patients (CS - 3000 Fenwall - 30; Amicus Baxter/Fenwall - 15; Cobe Spectra - 25). All procedures related to the harvesting and transplantation were performed in the Transplant Unit of the L. Turcanu Emergency Hospital for Children, Timisoara.

Collected peripheral blood progenitor cells were cryopreserved in autologous plasma and 10% DMSO

using controlled – rate freezing and stored in liquid nitrogen until thawing for reinfusion in patients.

We analysed the CD34+ cells number, MNC number, absolute T cell number and T cell subpopulation in the graft.

Hematological analysis

Blood samples were obtained 4 times/year in the first year and than 2 times/year post autologous peripheral stem cell transplant. A three-part differential was performed by using a Coulter Counter according to the manufacturer’s instructions.

Immune phenotyping

Lymphocyte subsets were monitored by using flow cytometry. All analyses were performed on an Becton Dickinson flow cytometer adjusted according to the manufacturer’s instructions. One hundred l blood was incubated with fluorochrome-conjugated (FITC/PE) primary antibodies for 15 min at RT in the dark. Anti-CD3, anti-CD4, anti-CD8, anti-CD45 antibodies were used for T lymphocyte phenotyping, anti-CD19 for B lymphocytes and anti-CD16/56 for NK cells. Background fluorescence was determined with appropriate isotype-specific controls. Lymphocytes were gated using linear forward and side scatters to exclude non-lysed erythrocytes and cell debris. The following antibodies, CD45/CD4-FITC, CD45/CD8-FITC, CD45/CD8-FITC, CD45/CD4-FITC were purchased from BD Pharmingen and used in a concentration of 20 l.

Statistical analysis

Correlation between absolute cell numbers in peripheral blood progenitor cells and peripheral blood T cells was tested by Pearson’s correlation coefficient.

RESULTS

The median administered quantity of CD 34+ cells was $5.63 \pm 7.01 \times 10^6 / \text{kg}$ and $3.13 \pm 2.38 \times 10^8 / \text{kg}$. for mononuclear cells MNC. Median lymphocyte T administration was $16.59 \pm 10.78 \times 10^7 / \text{kg}$ for CD3 lymphocytes, $4.59 \pm 2.45 \times 10^7 / \text{kg}$ for CD3/CD4 lymphocytes, $4.21 \pm 3.57 \times 10^7 / \text{kg}$ for CD3/CD8 lymphocytes, $1.16 \pm 1.89 \times 10^7 / \text{kg}$ CD56/CD16 for NK.

The cohort included 23 patients aged under 18 years and 47 patients with age between 18 – 65 years. 70% of patients had multiple relapses or incomplete remissions. (Fig.1)

The indication for SCT (Stem Cell transplantation) was in 50% of the patients Hodgkin lymphoma, in 18.57% non-Hodgkin lymphoma, in 10% multiple myeloma, whereas ,acute lymphoblastic leukemia, acute myeloid leukemia, PNET, Ewing’s sarcoma, rhabdomyosarcoma, neuroblastoma and germ cell tumor represented the malignancy in 21.43% of patients. (Fig.2)

Median engraftment time was 14.95 ± 6.06 days for granulocytes and 19.73 ± 10.95 days for thrombocytes.

The lymphocyte B (CD19+) recovery was 13% compared to the normal values for the correspondant age groups at day 30+ with full recovery at 3 months following transplantation. (Fig.3)

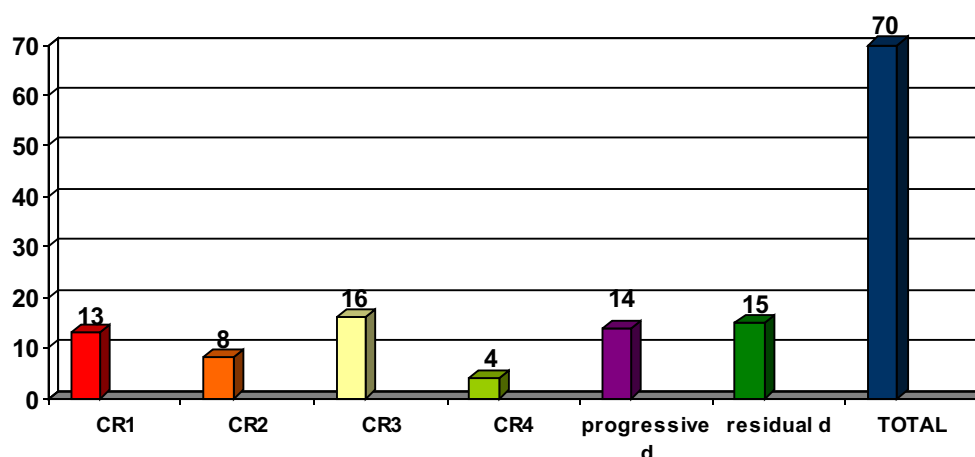


Fig. 1 – Disease stage at the moment of peripheral stem cell apheresis. CR1=complete remission 1; CR2= complete remission 2; CR3 = complete remission 3; CR4 = complete remission 4; progressive d.= progressive disease; residual d= residual disease

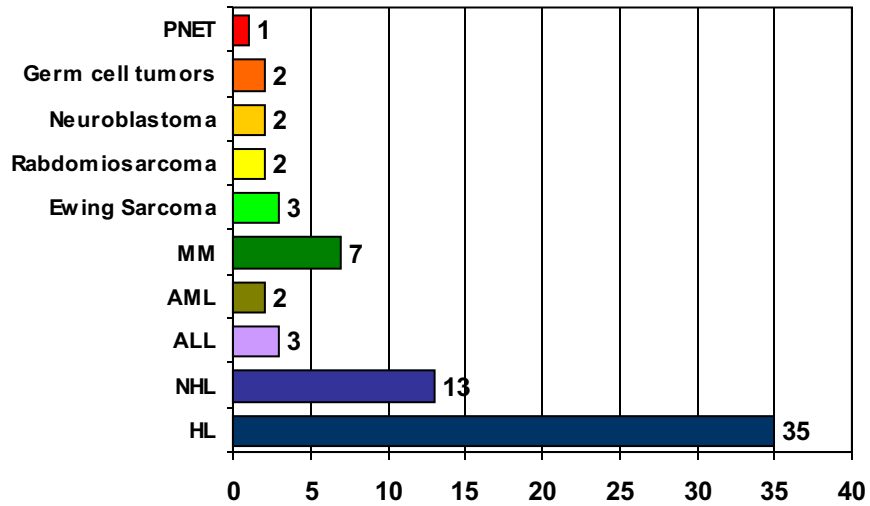


Fig. 2 – Autologous HSCT for various malignancies (a).MM= multiple myeloma; AML= acute myeloid leukemia; ALL= acute lymphoid leukemia; NHL= non-Hodgkin Lymphoma; HL= Hodgkin Lymphoma

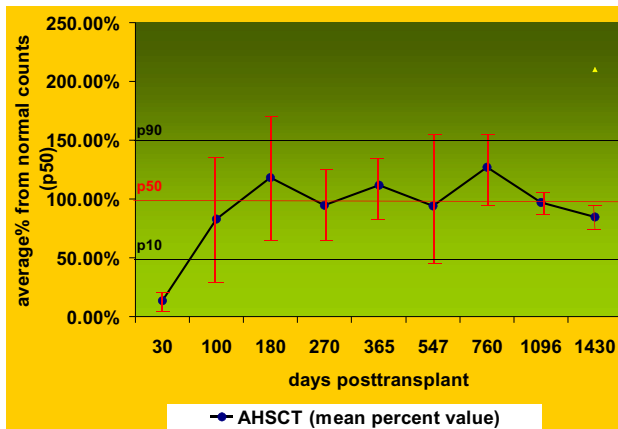


Fig. 3. Percentual median values of B lymphocytes CD19+ compared to absolute normal values for the respective age group (p50)

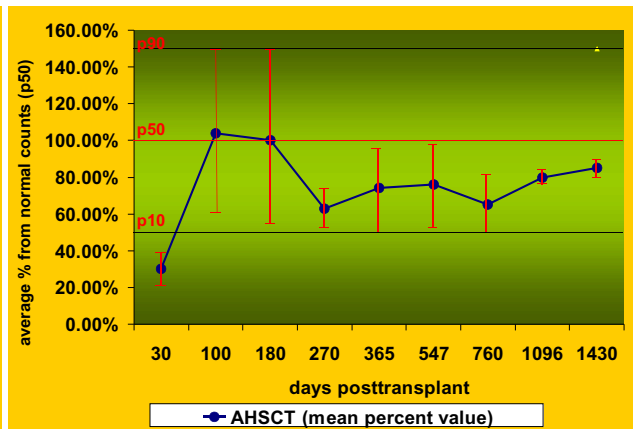


Fig. 4. Percentual median values of T lymphocytes CD3+ compared to absolute normal values for the respective age group (p50)

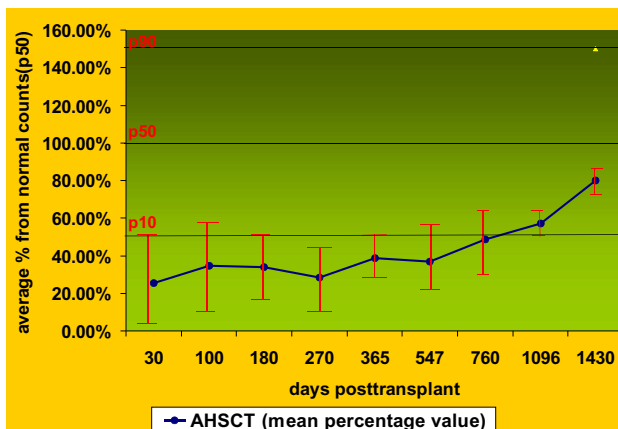


Fig. 5. Percentual median values of T lymphocytes CD3+ CD4+ compared to absolute normal values for the respective age group (p50)

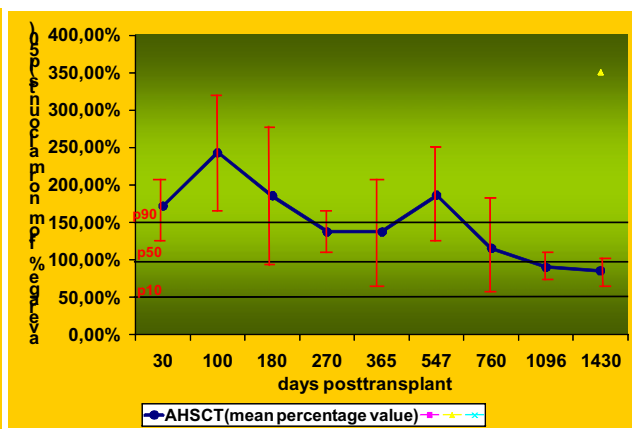


Fig. 6. Percentual median values of lymphocytes CD3+ CD8+ compared to absolute normal values for the respective age group (p50)

The mean percentage for lymphocyte T (CD3+) recovery at day 30+ was 30%. The reconstitution dynamics followed an ascending trend but the absolute

values remain under the normal limits even at 4 years after transplantation. (Fig.4)

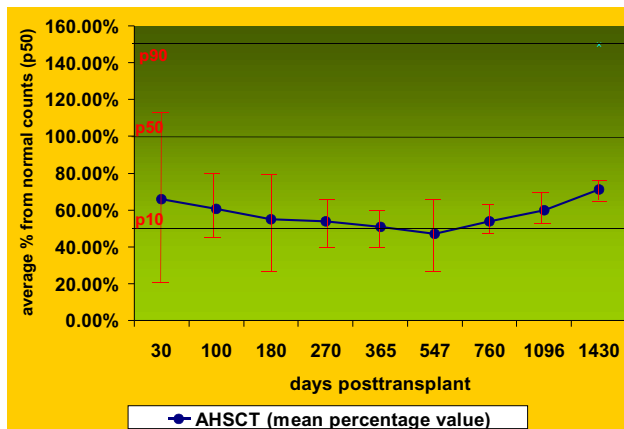


Fig. 7. Percentual median values of lymphocytes CD3-CD56+/CD16+ (NK) compared to absolute normal values for the respective age group (p50)

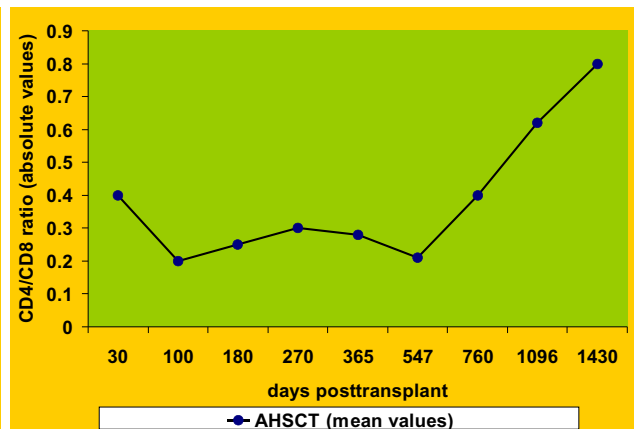


Fig. 8. CD4+/CD8+ ratio dynamics – mean values

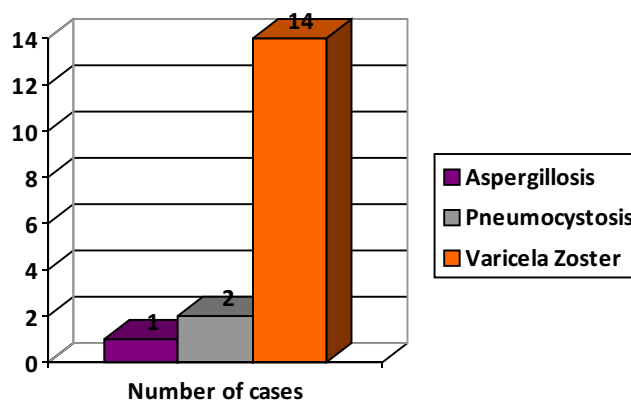


Fig. 9 Immunodeficiency related complications

Lymphocyte (CD3+CD4+) recovery at day 30+ 25,35%, ascending in dynamic but with a only 57% recovery at 4 years after transplantation. (Fig 5)

At day 30+ the mean percentage value for lymphocyte (CD3+CD8+) was 171,25%. This markedly increased values maintained until day 270+. (Fig.6)

NK cells (CD3-CD56+/CD16+) presented a recovery of 66% at day 30+ sustained at this mean percentage value even at 4 years post transplant. (Fig.7)

Low CD4+/CD8+ ratio caused by a persistent increase of CD8+ lymphocytes and a constant reduction of CD4+ lymphocytes was present even at 4 years after transplantation. (Fig.8)

One patient (1.42%) developed pulmonary Aspergillus infection at six months post transplantation. Two cases (2.85) of Pneumocystis carinii pneumonia were diagnosed after day 100+ post transplantation and 14 reactivations (20%) of the varicella zoster virus.(Fig.9)

No significant correlation was observed between the graft composition regarding quantity of CD34+infused cells or infused T cells and the immune reconstitution.

DISCUSSION

Immune reconstitution involves several components of the immune response. These include reappearance of functional B cells, thymic and extra-thymic T-cell development, reconstitution of effector cells including cytotoxic T cells and natural killer (NK) cells, and efficient antigen presentation to reconstitute the pretransplantation immune repertoire.

The relative and absolute numbers of circulating cells expressing CD19 and CD20, two markers of mature B cells, are decreased during the first 3 months following transplantation. [9,10] After this period, the numbers of such cells increase to a plateau at 6 to 9 months. In the first year following engraftment the fact that the majority of circulating B cells carry the CD23+, CD38+ undifferentiated phenotype [10] suggests that the majority of posttransplantation circulating B cells are poorly differentiated. Deficiencies in humoral responsiveness in HSC recipients is attributed to both decreased T-cell help and to intrinsic B-cell defects.[11] Recovery of in vivo B-cell function demonstrates a selective defect with

normal serum levels of IgM returning at 6 months, IgG at 12 months, and IgA after 2 years, reflecting a recapitulation of normal B-cell development. [12]

After autologous peripheral stem cell transplant, the relative number of CD3+ cells is significantly decreased compared with those of normal controls during the first month postgrafting, returning to normal levels within 3 months.[13] In addition, a decrease in the relative and absolute numbers of CD4+ cells in the peripheral blood is commonly seen and can persist for a year or more.[14] In contrast, the relative and absolute number of CD8+ cells reconstitutes fairly rapidly resulting in an inverted CD4/CD8 ratio in the months following autologous transplantation.[15]

Following HDC, the restoration to all levels of the hematopoietic hierarchy is thought to be based on regenerative potential of pluripotent HSCs transfused within the graft.[6-9] In the past, sustained engraftment of bone marrow was empirically correlated with the total number of nucleated cells transfused.[1-4] There is accumulating evidence for the predictive power of CD34+ cell concentration per kg body weight (BW) leading to sufficient engraftment.[2,3] The threshold of 2×10^6 CD34+ cells/kg BW is established. Increasing stem cell dosages are correlated with an accelerated speed of hematopoietic reconstitution.[16]

The recovery of lymphocyte subsets differs for B, T and NK cells, reflecting different pathways used by lineage-committed precursors to mature into various terminally differentiated cells during their developmental progression.[17] A primary increase of CD3+CD8+ cytotoxic T lymphocytes could be detected, whereas the CD3+CD4+ helper/inducer T cells remained depressed throughout the study period. The early engraftment might more likely be attributed to the transfusion of T cells whose life-span in the recipient is not well known. In addition, the survival of endogenous T lymphocytes in

spite of HDC could not be ruled out. Nevertheless, the life-long reconstitution of the cellular immune system needs the generation of T lymphocytes from HSCs. [18, 19]

Our results for autologous HSCT patients are similar to those already published in the specialized literature. However, the deficiencies of the investigated lymphocytic subpopulations were more severe and prolonged. Reconstitution dynamics was characterized by an initial global immune depression in the first 30 days following transplantation with a mean percentage value of under 30% when compared to the normal values for the correspondent age groups for CD19+, CD3+, CD3+4+ cells. The mean percentage values for NK cells and CD3+CD8+ T cells was 55% on day 30+ following transplantation.

After day 30+ immune reconstitution dynamics followed a clearly ascending trend. However the absolute values for the investigated lymphocytic subsets remained under the normal limits even at 4 years after transplantation. The exception to this pattern was represented by the CD19+ population which normalized at 3 month following transplantation and the CD8+ population which showed markedly increased absolute values from day 30+ to day 270+.

CONCLUSIONS

Post transplantation global immunodeficiency in our patients was more severe and more prolonged compared to published data, probably due to the intense regimens administered prior the HSCT. All patients had a low CD4+/CD8+ ratio during at least the first year post-transplant, caused by a persistent increase of CD8+ lymphocytes and a constant reduction of CD4+ lymphocytes, making the patients susceptible to infections for a prolonged period of time post-transplant.

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