etiologic agent was found, so it was considered that AA was secondary to HIV. Treatment was initiated with D4T (Stavudine), 3TC (Lamivudine) and Efavirenz achieving undetectable viral load and increasing CD4 count. Patient also received Erythropoietin (EPO) and granulocyte-colony stimulating factors (G-CSF) showing an increased number of white blood cells (WBC) but continued with high transfusional requeirment. We had to stop treatment in October because of liver failure and lactic acidosis. In November we changed Stavudine for Tenofovir and reiniciated treatment. Viral load was always undetectable and CD4 count was > 500 cells/ul. However megakaryocytopoiesis and erythropoiesis did not respond, requiring many transfusions. Coombs Direct Test (CDT) and Coombs Indirec Test (CIT) were positive.He showed an immunohaematologic profile with 1 autoantibody (Anti-e) and 3 alloantibodies (Anti-Jka, Anti-Lua, Anti-Cw).

As he had an identical twin, he was submitted to a syngeneic BMT. Although he was heavily transfused (421 Units), we did not want to increase immunosuppression so as not to have viral reactivation. The conditioning regimen consisted of Cy 50 mg/kg/qd x 4, and in order to lower the risk of engrafment failure, peripheral blood stem cells were used to maximize the number of donor cells infused (11×10^6 CD34+ cells/kg). At the transplantation the patient was in high-risk. The antiretroviral treatment was not discontinued. No graft versus host disease (GVHD) profilaxis was needed. Neutrophils and platelets engrafted at day +11.

After 10 months of transplantation he continues in complete haematologic remission. Serum antibodies, CDT and CIT are negative.Viral load remains undetectable (b-DNA).

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ABSOLUTE CHIMERISM AS A TOOL IN MONITORING IMMINENT AND MANIFEST GRAFT REJECTION AFTER HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING

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Graft rejection after hematopoietic cell transplantation (HCT) with nonmyeloablative conditioning is a rare event and a serious clinical problem. Manifest (N=8) and imminent rejection (N=4) in a cohort of 98 consecutive patients with hematological malignancies were analyzed. The patients were conditioned with fludarabine 30 mg/m^2 and 2 Gy of total body irradiation and transplanted with peripheral blood stem cells. Intervention aiming at reversing imminent rejection with donor lymphocyte infusion (DLI) alone or preceded by immunosuppression with pentostatin was attempted with highly variable results. Chimerism analysis is the standard method to monitor engraftment and rejection. In the present report we have evaluated the product of absolute T cell counts and chimerism, which we have termed absolute chimerism, for monitoring patients with manifest or imminent rejection. The results suggest that recipient T cell counts > donor T cell counts and increasing recipient T cells post-transplant are risk factors for rejection. Peaks of absolute recipient CD4+ and/or CD8+ T cell counts were seen in relation to rejection and peaks of donor CD4+ and/or CD8+ T cells were seen in connection with acute graft-versus-host disease. Furthermore absolute chimerism plots in some cases clearly indicate the time-interval where disappearance of recipient T cells takes place. These findings may be of importance for understanding the cellular mechanisms underlying alloreactivity. Absolute chimerism plots point to a variable immunosuppressive effect of pentostatin on recipient T cells as explanation for pentostatin/DLI-failure in reversing rejection. We conclude that absolute chimerism plots can contribute significant new information of value for routine monitoring of patients with mixed chimerism as well as for research purposes. Following rejection patients are at risk of dying from infections and progression/relapse of their malignancy. Retransplantation is feasible and well tolerated after HCT with nonmyeloablative conditioning. In patients with imminent graft rejection retransplantation is an attractive alternative to DLI or immunosuppression/DLI.

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AN IMPROVED METHOD FOR ENGRAFTMENT MONITORING USING REAL-TIME PCR

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The commonly used engraftment monitoring method of short tandem repeat (STR) amplification following allogeneic hematopoietic stem cell transplantation (HSCT) is hampered by several shortcomings affecting sensitivity, accuracy, reproducibility, and results interpretation. In an effort to provide a better solution, we have developed a truly quantitative, real-time PCR method. Real-time PCR technology is ideally suited for this application because it is highly sensitive and quantitatively much more accurate and precise than STR analysis. It is not affected by problems inherent to STR amplification and analysis such as plateau bias, preferential allele amplification, and stutter artifacts. With our method, genomic mixtures are easily resolved, and analysis is straightforward and automatable. Success and maintenance of allogeneic HSCT may be enhanced with such an improved engraftment monitoring method.

We have developed a panel of non-repetitive polymorphic markers, with representatives on all chromosomes, useful for identifying differences between recipient and donor pairs. Our statistical analyses given allele frequencies in multiple human populations demonstrate that with relatively high minor allele frequencies, a limited panel of markers can be assembled which would have high probability of providing informative markers that could distinguish HLA-matched individuals. The testing process first requires that the donor and recipient genomic DNA be screened in order to identify informative markers between the two genomes. Once defined, these markers are used post-transplant to quantify the relative percentage of recipient genetic material in the donor background. Compared to the current STR assays, our method shows a greater than 100-fold increase in sensitivity with excellent accuracy and precision at concentrations as low as 0.01% recipient in a background of 99.99% donor DNA. Given the reproducibility of results and ease of data analysis, this approach provides the means for standardization within a testing lab as well as between testing centers, which cannot be accomplished with homebrew and off-label methods such as STR analysis. From a clinical perspective, improved sensitivity, accuracy, and precision provide the potential to detect disease relapse or transplant rejection at a much earlier stage. This information may be useful for improving decisions related to maintenance treatments such as GVHD prophylaxis and/or donor lymphocyte infusion.

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CHARACTERISTICS OF TWO CONDITIONING REGIMENS CYCLOPHOSPHAMIDE PLUS ANTITHYMOCYTE GLOBULIN VERSUS CYCLOPHOSPHAMIDE PLUS BUSULFAN IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

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We report a retrospective study of the clinical features and outcome for 32 allogeneic stem cell transplants (AlloSCT) from HLA-matched sibling donors in severe aplastic anemia conditioned with 200 mg/kg of cyclophosphamide plus 90 mg/kg of horse antithymocyte globulin (ATG) in 17 patients and 120 mg/kg of cyclophosphamide plus 12 mg/kg of busulfan (Bu) in 15 patients, at Hospital São Paulo and Hospital Santa Marcelina, from November 1993 to August 2003. Considering high cost of ATG and the difficulty to obtain it for the majority of our centers, the objective was to compare clinical variables as age, sex, engraftment, interval of time from diagnosis to AlloSCT, number infused of total nuclear cells, previous transfusions, occurrence of acute and chronic GVHD, infections, acute and late graft rejection and overall survival, between these two conditioning regimens. We analyzed the long-term hematopoietic chimerism by FISH, using a

double XY probe, in sex-mismatched pairs, and VNTR, using PCR with Apo-B, D1S80, vWF and DXS52 primers in all patients. Mucositis and acute GVHD were more evident in the Bu group. Late rejection occurred specially in the ATG group. All the others variables and overall survival were similar between these two conditionings. By FISH evaluation, there were complete chimerism in 8 of 10 patients and there was a case of mixed chimera and another of autologous reconstitution. VNTR determined chimera in 15 of 17 donor/ recipient pairs (88.2%). Complete chimerism, analyzed by all primers were seen in 8 of 17 (47%) patients. Mixed chimerism and autologous reconstitution patients, observed by FISH, were confirmed by VNTR just using D1S80 and vWF primers, respectively. We conclude that both conditioning regimens were effective and the analyzed clinical data were comparable between the groups, however, a larger number of patients need to be studied in order to establish the best conditioning. The methods for evaluation of the chimera are sensitive and informative using VNTR and FISH methodologies.

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EARLY EXPANSION OF LYMPHOID CELLS PRECEDES MYELOID ENGRAFTMENT FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION USING TRULY NONMYELOABLATIVE CYCLOPHOSPHAMIDE/FLUDARABINE CONDITIONING

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Little is known regarding the mechanism of engraftment of allogeneic cells in humans, partially because there are few cells to analyze in the early (first 2 weeks) post-transplant period. After myeloablative conditioning, monocytes are the initial donor cells identified in recipients' blood, followed by polymorphonuclear leukocytes and then lymphocytes.

Forty-nine consecutive patients with hematological malignancies (median age, 55 years) received PBMCs from a matched related (MRD) or unrelated (MUD) donor following cyclophosphamide, 60 mg/kg on days –6 and –7 (total dose 120 mg/kg) and fludarabine, 25 mg/m^2 for 5 consecutive days (day –5 through day –1; total dose, 125 mg/m^2). GVHD prophylaxis consisted of cyclosporine (n=33) or cyclosporine + mycophenolate mofetil (n=16). Acyclovir, fluconazole and quinolone prophylaxis were provided and freshly harvested, non-manipulated PBMCs were infused within 24 hours of collection. One patient with chronic lymphocytic leukemia (CLL) and a pre-transplant lymphocyte count of >150,000/mm^3 (considered an outlier) was removed from the analysis. A database (Excel®, MS, Redwood, CA) was utilized to record white blood counts (WBC) obtained from the computerized medical record; each patient's total WBC, neutrophil, lymphocyte, and monocyte percentages were recorded from day –7 to day +30. Cumulative data were plotted using S-Plus® software (Insightful, Seattle, WA) and median times to peak percentages were determined. Patients who received conventional conditioning and similar grafts prior to transplantation for hematological malignancies (AML or MDS) over a similar time period (n=46) were studied and engraftment patterns compared.

The following phenomena were observed after nonmyeloablative transplantation: (1) no ¨ bump¨ , (increase in the peripheral WBC) the day following cell infusion; (2) resolution of neutropenia at a median of 12 days after MUD transplants and 15 days after MRD transplants (p=0.778); (3) median peak lymphocyte, monocyte and polymorphonuclear percentages occurred 9, 12 and 23 days post-infusion, respectively.

Early disappearance of infused cells from the circulation and relative expansion of lymphocytes preceding the emergence of monocytes and polymorphonuclear cells suggests that relatively quick engagement of donor cells by the marrow microenvironment is followed by an immunologically active process after truly nonmyeloablative cyclophosphamide/fludarabine conditioning.

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NON-MYELOABLATIVE CONDITIONING THERAPY WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND ATG ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM HLA-IDENTICAL SIBLING DONOR IN PATIENTS WITH SEVERE APLASIC ANEMIA (SAA)

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Allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling is a curative form of therapy for patients with acquired severe aplastic anemia.

Survival has significantly improved over the past 3 decades. The actuarial risk of rejection has been reduced to about 7%. Improved results with survival in excess of 90% have been reported. Current preparative therapies are associated with early and late sequelae such as acute and chronic graft-versus-host disease (aGvHD or chGvHD, respectively) and secondary tumors. In two patients (6 years and 11 years old) with SAA, who had an HLA-identical sibling donor, but could not proceed with myeloablative therapy at the time of transplant for various reasons (delay in results of chromosome stability and fragility in one patient and abnormal pulmonary function in the second), had a non-myeloablative preparative regimen with Fludarabine (30 mg/m^{2}x4 doses) Cyclophosphamide (5 mg/kgx4 doses) and rabbit ATG (1.5 mg/kgx4 doses) followed by an unmanipulated allogeneic BMT. Graft versus host disease prophylaxis consisted of Cyclosporine from day -1 and Methotrexate 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, +11 after transplant. Myeloid engraftment occurred on day +15 and day +28. The time to a platelet count >20,000 unsupported was +11 days and +29 days. No transplant-related toxicities, including mucositis or alopecia, were recorded. There were no signs for aGvHD or chGvHD. The patients continue with full donor chimerism 31 months and 6 months post transplant, respectively. This data suggests that a non-myeloablative, immunosuppressive regimen is sufficient to provide a stable engraftment in patients with SAA. This approach may be associated with decreased transplant-related short- and long-term toxicities. A larger study is needed to fully evaluate the outcome and toxicities profile associated with this conditioning.

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INFLUENCE OF INTERLEUKIN-6 (IL-6) GENE POLYMORPHISM ON THE OUTCOME OF PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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BACKGROUND: IL-6 is an important mediator of inflammation and its production depends on the functional IL-6 gene polymorphism (IL-6-174*G/C). Allele G expression is associated with higher IL-6 production. The polymorphism of recipient and/or donor might influence immunological reactions after allogeneic stem cell transplantation (SCT), particularly graft vs. host disease (GvHD) and graft vs. tumor (GvT) one.

AIM OF STUDY: To assess the influence of recipient/donor functional IL-6 gene polymorphism on the development of acute/chronic GvHD, tumor relapse and mortality.

PATIENTS AND METHODS: 56 patients were allografted from HLA-identical related donor. 54 recipients (96%) underwent the procedure because of incurable hematological malignancy and 33 ones (59%) after reduced intensity conditioning (RIC). IL-6-174* genotyping of recipients/donors was provided by the use of polymerase chain reaction with sequential specific primers (PCR-SSP). The influence of GvHD development, tumor relapse and mortality on the IL-6-174*G/C allele manifestation in recipients/donors was assessed by the methods of univariate as well as multivariate statistical analysis.

RESULTS: Statistical analysis did not confirm the significant influence of functional IL-6 gene polymorphism of recipients/