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/