patients who received the pharmacological therapy plus Extracorporeal radiopheresis twice a week. Apoptosis induction, phenotype of dendritic cell subsets and cytokine production were evaluated *in vitro*. *Results.* 15 patients with aGVHD and 15 with cGVHD were studied. In aGVHD grade IV neither treatment affected the long-term survival. Nevertheless, in 3/4 patients (75%) who received radiopheresis, diminution of clinical symptoms (gastrointestinal bleeding and skin rash) was observed since the first week of treatment, improving the quality of life. This effect was also observed at later in patients receiving only pharmacological therapy. In cGVHD, 7 patients received radiopheresis, 5 (71.4%), improved the skin pain since the first week and the skin sclerosis after 6-12 months. In one patient, control of cGVHD progression was obtained. Four of these patients had previously received pharmacological therapy without control of the GVHD. 1/8 patients that received only pharmacological therapy, three improved (37.5%), in three (37.5%), were control of cGVHD progression and two got worse (25%). Histological skin follow-up showed that in aGVHD severity score were one grade lower on all radiopheresis cases evaluated. In patients that received pharmacological therapy only, 80% were the same grade, 20% got worse. For cGVHD skin biopsies were made after 6-12 months. Lower or same histological grade were observed in six patients who received radiopheresis and four patients with only pharmacological therapy, 87,5% and 50% respectively. Induction of apoptosis in cells that received radiopheresis was evaluated with DIOC-6/BE. No changes in the phenotype of dendritic cells differentiated from monocytes with IL-4+GM-CSF, were observed. *Summary*: Better clinical and histological therapeutic effects were observed on patients who received Extracorporeal Radiopheresis. Multicentric studies will contribute to evaluate this therapy in a larger number of patients.

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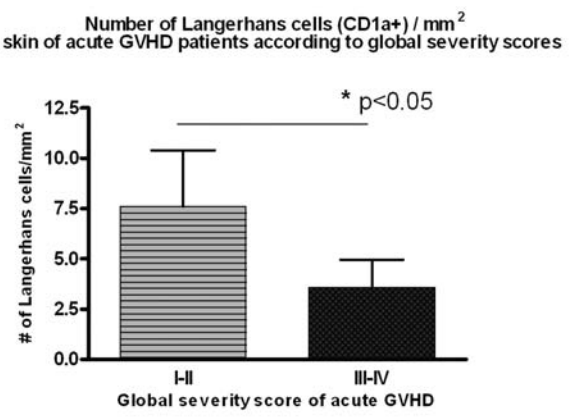
0826

IMMUNOHISTOCHEMICAL EVALUATION OF INFILTRATING CELLS (CD4+, CD8+, AND CD56+) AND LANGERHANS CELLS IN SKIN OF GRAFT VERSUS HOST DISEASE PATIENTS

M. Velasquez-Lopera,[1] A. Vargas,[1] G. Arellano,[1] L.A. Correa,[1] J.C. Wolff,[1] G. Sanclemente,[1] J.C. Villamizar,[1] A. Jaramillo,[2] L.F. García,[1] F. Cuellar-Ambrosi[1]

[1]*Universidad de Antioquia, Medellin, Colombia;* [2]*Rush University Medical Center, Chicago, USA*

Graft vs. host disease (GVHD) occurs in about 60-80% of allogeneic hematopoietic stem cell transplant recipients due to mismatching of major and minor histocompatibility antigens. Cutaneous involvement is the most frequent clinical manifestation of GVHD. In this regard, Langerhans cells (LC) have been shown to play an important role in the pathogenesis of GVHD as antigen-presenting cells through both the direct and indirect allo-recognition pathways. Thus, we carried out an immunohistochemical study to determine the characteristics of the infiltrating T cells (CD4+ and CD8+) and NK cells (CD56+) as well as LC (CD1a+) on skin biopsies of GVHD patients and their correlation with global severity scores. Forty-two patients were allo-transplanted between June, 1998 and December, 2002. Twenty-nine (69%) patients developed GVHD; among these, 15 (36%) developed acute GVHD, 5 (11.9%) developed chronic GVHD, and 9 (21.4%) developed acute and chronic GVHD. Immunohistochemical enumeration of CD1a+, CD4+, CD8+, and CD56+ cells were performed in paraffin-embedded punch skin biopsies taken mainly from the thorax. Among the 24 cases with acute GVHD, skin involvement was observed in 23/24 (95.8%) patients, most of them with G I-II scores. Intestinal GVHD was observed in 20/24 (83.3%) patients with 15 (75%) patients with G I-II scores and 5 patients (25%) with G III-IV scores. Hepatic GVHD was observed in 8 (33.3%) patients, 5 (62.5%) of those patients with G I-II scores. The number of LCs/mm2 in dermis and epidermis was significantly lower in cases with major global severity scores: Normal skin donors: (mean±SD) 15.6±1.6, acute GVHD G-I-II: 7.5 ± 8.8 and G-III-IV: 3.6±2.7 (p<0.05). An increase in the ratio of infiltrating perivascular and epidermal CD8+ T cells was observed and it was inversely proportional to the number of LCs on epidermal and dermal layers of the skin. There was no increase of CD56v NK cells in patients with acute GVHD as compared to normal controls. Figure 1. Extensive chronic GVHD was seen in 7/14 (50%) patients. The number of LC was similar in limited and extensive chronic GVHD (9.5±4.2 and 9±12.7, respectively). In *de novo* chronic GVHD, the number of LCs was higher (13.2±4.6), than in progressive (7.3±0) or quiescent (2.7 ± 3.9) GVHD (p=0.05). Scleroderma-like presentation showed higher number of LC (9.7±6.7) as compared to lichen-like lesions (7.3±0).



No increase in the ratios of infiltrating epidermal and perivascular CD8+, CD4+ or CD56+ cells was observed. In summary, in acute severe systemic GVHD, a significantly lower number of LCs and higher number of CD8+ T cells were observed. These changes were not observed in chronic GVHD. This study indicates that skin CD8+ T cell/LCs ratios could be used as an additional tool for diagnosis and follow-up of GVHD.

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DYNAMICS OF LINEAGE-SPECIFIC CHIMERISM IN PATIENTS AFTER NON-MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION

O. Horky, J. Mayer, M. Borsky, J. Pospisilova, M. Krejci, D. Dvorakova

Faculty Hospital Brno, Brno, Czech Republic

Backgrounds. Monitoring of engraftment by assessing chimerism has become routinely used method after allogeneic hematopoietic stem cell transplantation (HSCT). Information about the proportional degree of donor and recipient hematopoiesis and its dynamics in time is particularly important in patients receiving non-myeloablative preparative regimen. *Aims.* The purpose of this study was to describe some aspects of correlation between persistence of lineage-restricted mixed chimerism (MC), complete donor chimerism (CDC), minimal residual disease (MRD), risk of graft versus host disease (GvHD) and relapse. We retrospectively analyzed data of chimerism obtained from 21 patients who underwent non-myeloablative HSCT for chronic myeloid leukemia (CML) in our centre between June 1998 and December 2005 (827 chimerism quantification of whole blood samples, 259 lineage-restricted ones). The conditioning regimen consisted of fludarabine, busulfan and ATG Fresenius. GvHD prophylaxis was cyclosporine A with or without MMF. *Methods.* Assessment of chimerism was carried out by singleplex amplification of variable number of tandem repeat (VNTR) or short tandem repeat (STR) regions - mainly with capillary electrophoresis with fluorescence detection (apart from the beginning when densitometry was involved). Since September 2005 real-time quantitative polymerase chain reaction (RQ-PCR) of insertion/deletion polymorphism was adopted. Detection of MRD was performed by competitive nested PCR and by reverse-transkriptase RQ-PCR. Individual leucocyte subsets (B cells, T cells, NK cells, granulocytes and monocytes) were fluorescence-activated cell sorter (FACS)-sorted according to corresponding antigens. *Results.* Observed patterns are mentioned bellow: 1) MC in whole blood along with MRD negative samples always means that autologous population comes from a lymphoid line. 2) MRD positive samples correlate with appropriate MC in granulocytes. 3) MRD positive samples along with CDC in whole blood are caused by limited sensitivity of VNTR-/STR- PCR (repeatedly retrospectively verified by RQ-PCR, which showed microchimerism). 4) Achievement of CDC is preceded with CDC in T-cells. However CDC in T-cells is not necessarily followed by CDC in whole blood (failure of graft versus leukemia (GvL) effect). 5) MC (even in T-cells) does not always means protection from GvHD. *Conclusions.* Our preliminary data demonstrate that lineage-restricted chimerism analysis allows better understanding of hematopoiesis recovery after HSCT and can significantly contribute to interpretations of relations between chimerism and MRD or GvHD. On the other hand, however, the predictable value of chimerism and especially microchimerism must be further investigated.