EVALUATION OF FLOW CYTOMETRIC RETICULOCYTE MATURITY INDEX (RMI) AS A DIAGNOSTIC AND PROGNOSTIC INDICATOR IN ACCIDENTAL TOTAL BODY IRRADIATION

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INTRODUCTION

In cases of accidental total body overexposures early diagnostic and prognostic methods are recquired. After high doses, bone marrow could be severely damaged. Between peripheral blood cells, reticulocytes could be considered as a useful indicator of erithropoietic activity to evaluate the extension of damage to haematopoietic system and to predict bone marrow recovery.[1]

After an accidental total body irradiation (TBI), their presence in peripheral blood, even in a very low quantity, indicates a remainder bone marrow activity. Their increase after a bone marrow aplasia, may be considered as a sign of functional recovery.

Flow cytometric analysis provide an objective measure of reticulocyte maturity, since fluorescence intensity is directly proportional to ribonucleic acid (RNA) content [2]. It is thus possible to derive a reticulocyte maturity index (RMI) taking into account the fraction of reticulocytes with high fluorescence intensity (HFR) which represents the subset at the earliest maturative stage .

With the purpose of testing the usefulness of flow citometric reticulocyte analysis with determination of a RMI as an early indicator of radioinduced marrow suppresion, this method was assayed in an animal model of TBI.

Considering that therapeutical irradiations could provide useful models of human accidental irradiations,similar studies were performed in fourteen patients undergoing TBI as a conditioning regime for bone marrow transplantantion in order to evaluate the clinical applicability of this method for monitoring bone marrow functional recovery.

MATERIALS AND METHODS

Animal studies: Wistar rats were irradiated with Co 60-gamma radiation, at doses of 2 Gy, 4 Gy and 6 Gy . Blood samples were taken from the tail vein on days 0, 1, 3, 7, 10, 17.and 30 after irradiation (a.i.). Similar sampling was carried out with non-irradiated animals.

*Clinical studies:*fourteen patients undergoing bone marrow transplantation (BMT) (6-54 years) were enrolled for this study. Pretransplant conditioning regimes consisted in chemotherapy (started on day -9) plus radiotherapy (between days -4 and -1). Bone marrow infusion was performed on day 0. TBI was administered at 3 Gy/day in twice daily fractioned doses, at a dose rate of 0.04 Gy/min. Blood samples were obtained daily during conditioning treatment and three times weekly until patient's discharge.

Flow cytometry of reticulocytes: all blood samples using EDTA as anticoagulant were treated in the same way. Whole blood (5uL) was stained for flow cytometric analysis with thiazol orange (TO) Retic Count® (Becton Dickinson) and the corresponding unstained sample was used as autofluorescent control. Both samples were analyzed on a Facstar Plus® cell sorter (Becton Dickinson).

The fluorescence of 50000 red cells was collected. The reticulocyte percentage and the mean fluorescence intensity were calculated using the Lysis I software program.

For RMI defining , data analysis was performed by two different protocols. For the first one, mean channel number of fluorescence was expressed as arbitrary units of fluorescence (UAF/RMI expression) [3]. For the second method, fluorescence intensity of reticulocytes was divided into regions of low (LFR), moderate (MFR) and high fluorescence (HFR) according to Davis [4] [5] and HFR %

was derived by dividing the number of reticulocytes in the high fluorescence area by the total number of reticulocytes.

Engraftment monitoring in BMT patients: a post-transplantation increase in absolute neutrophil count (ANC) to > 500/ml in two consecutive samples was employed to define myeloid engraftment. The second succesive increase of platelets count (PC) to > 25000/ml was considered as indication of trombopoietic recovery. Erithropoietic recovery was defined by three succesive rising counts in RMI following the nadir post-transplantation .

Statistical analysis: satistical analysis of data was performed using the Wilcoxon matched pairs signed rank sum test and one way Anova test.

RESULTS

Animal studies: Considering the whole groups, total reticulocyte percentage fell progressive to a nadir on day 3 a.i. (5.56 % from basal values , $p < 0.01$) A slow recovery was then observed There was a rise on day 17 a.i. This rise was more evident in groups irradiated with the higher doses. In most animals basal values were recovered on day 30 a.i. There was a significant reduction in AFU/RMI on day 3 a. i. (day 0 vs day 3 : 41.1 +/- 5.71 vs 30.83 +/- 1.77 $p < 0.01$) indicating a lower RNA content in reticulocyte population. Recovery was significantly earlier in the group irradiated with 2 Gy ($p < 0.05$). A rise was evident in all groups on day 17 a.i. (figure 1).

Clinical studies: in all patients HFR % decreased more rapidly than reticulocyte count and dropped to zero in most patients. After the aplasic period, the rise in HFR % preceded by several days the rise in total reticulocyte number. Engraftment was detected on day 16.5 +/- 3.2 post-transplantation (p.t.) by HFR % , on day 19.8 +/- 4.6 p.t. by ANC ($p< 0.05$) and on day 26.5 +/- 7.8 p.t. by PC ($p < 0.05$). Figure 2 shows temporal behaviour of total reticulocytes number and HFR % from the beginning of conditioning treatment until patients' discharge.

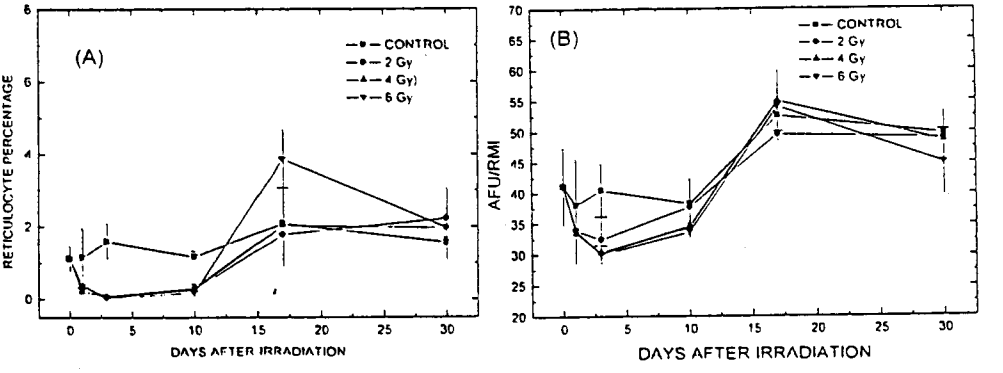

FIGURE 1 : Temporal behaviour of (A) reticulocyte percentage and (B) AFU/ RMI in total body-irradiated rats.

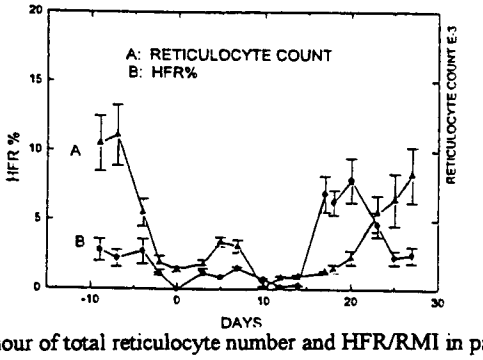

FIGURE 2 : Temporal behaviour of total reticulocyte number and HFR/RMI in patients undergoing bone marrow transplantatation Day 0: bone marrow infusion.

DISCUSSION

Reticulocyte count has been subject of numerous discussions regarding its clinical applicability and the new possibilities offered by flow cytometric methods. Besides the total number of these cells, the mean RNA content may play a role as a parameter of maturation stage , thus providing another useful tool for monitoring erythropoietic function.

Our data from animal studies indicate the sensitivity of this method for evaluating radioinduced damage to haematopoietic system, according to previous results of Tanke et al [6]. Early depletion in peripheral reticulocytes was evident in all irradiated animals but the results didn't reflect dependency to radiation doses . The percentage increased by the third week in all groups, including non-irradiated animals. A regenerative response from bone marrow to periodic blood loss could account for this late rise [7]. Higher values were reached in groups irradiated with higher doses. Thirty days a.i. basal values were recovered. Additional studies with other doses and dose-rates should be done to fully define dose-effect relationship. Considering AFU/RMI it has been possible to detect an earlier recovery in animals exposed to lower doses.

Our study with patients was primarily aimed on the early detection of bone marrow functionally recovery. Temporal relationship between erythroid, myeloid and megakaryocytic engraftment remains unanswered [3]. Although all peripheral blood cells ultimately arise from a totipotential haematopoietic stem cell, early regeneration of cell populations after BMT may be from commited progenitor cells. Hence, the rate of recovery of the three formed elements (red cells, white cells and platelets) could be independent of each other [1] Our results showed that bone marrow function was detected earlier by RMI than by either neutrophils or platelets Rise in HFR % preceded in about 3 days the rise in neutrophil counts This close temporal-relation is in good correlation with previous results of other similar studies [8]

Additional work will be required to standardize RMI and arrive at a common definition and calibration [2] Further experimental and clinical assays could provide more insight regarding the potential clinical utility of this method for monitoring accidentally overexposured patients.

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