Inflammatory response in radiation induced late effects

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Abstract

Late effects of radiation are generally irreversible and can have devastating effects on quality of life of people exposed either accidentally or during therapeutic radiation treatments. Although many etiologies have been suggested regarding these late toxicities, inflammatory parameters involved during the late phase are less known.
The aim of this study was to analyse the response of the immune system in the inflammatory reactions in patients with late skin injuries after radiotherapy or interventional fluoroscopy procedures. The expression of adhesion molecules ICAM1 and β1-integrin on granulocytes and lymphocytes, as well as changes in subpopulations of T lymphocytes and the level of C-reactive protein , a well- studied inflammatory marker were evaluated.
The follow up of twenty five patients, out of 160 referred to Burn Hospital from 1997, that showed late cutaneous reactions graded according to the RTOG / EORTC system is reported here.
The analysis of adhesion molecules expression revealed a higher expression of β1 Integrin on lymphocytes of Grade IV patients compared to non exposed controls. It was also noted a decrease in its expression values in the follow up of patients with good response to therapeutic treatment. This was paralleled by a tendency to a decrease in the T(CD4+) / T(CD8+) ratio of G4 patients with bad evolution compared to G4 patients with good evolution .
The parameters analysed, which require confirmation in a larger study , in combination with other inflammatory indicators, could be used as potential follow-up markers of the chronic radio-induced inflammation process just as its response to therapeutic treatments.

Key words: Radiation-induced late effects; Chronic inflammation; Immune response; Adhesion molecules.

1- Introduction

Up to now there are no established parameters for the follow-up of delayed radiation injuries [1]
Late toxicity is generally irreversible and can have devastating effects on quality of life of people exposed either accidentally or during therapeutic radiation treatments. They are the consequences of an imperfect tissue remodelling and of persistent radiation induced injuries [2]
Histologically, late manifestations of radiation damage include fibrosis, necrosis, atrophy and vascular lesions. Although many etiologies have been suggested regarding these late toxicities, chronic inflammation has been described as playing a key role.
The recruitment of leukocytes from circulating blood is decisive in the inflammatory reaction. All the steps in the recruitment cascade are orchestrated by cell-adhesion molecules (CAMs) on both leukocytes and endothelial cells, and different subsets of CAMs are responsible for different steps in extravasation. The involvement of CAMs in many inflammatory diseases has led them to be considered as targets for therapeutic interventions [3]. However the long term alterations of CAMs expression in irradiated tissues remain unclear
Otherwise, a link between chronic low-level inflammatory responses and alterations in homeostasis of immunity are still demonstrable in the blood of A-bomb survivors.. The most remarkable late effects of radiation were functional and quantitative abnormalities on T and B cells in survivors exposed to high doses [4]
The following study was conducted to examine the response of the immune system in the inflammatory reactions of patients with late skin injuries after radiotherapy (Rt) or interventional fluoroscopy procedures.
The expression of adhesion molecules ICAM1 and β1-integrin on granulocytes and lymphocytes, as well as changes in subpopulations of T lymphocytes and the level of C-reactive protein, a well- studied inflammatory marker were evaluated.

2. Materials and Methods

Patients

From 1997 to 2011 over 160 patients were referred to the Radiopathology Committee of Hospital de Quemados del Gobierno de la Ciudad de Buenos Aires (Burn Hospital) for the diagnosis and therapy of Cutaneous Radiation Syndrome. The follow up of twenty one patients that showed late cutaneous reactions graded according to the RTOG / EORTC system is reported here.
Median age (ranges): 63 (49-79) years.
Late effect was considered from three month after the radiation procedure.
The study was approved by the Research and Ethics Committee of Burn Hospital. Informed consent was obtained from all patients.

Sample Collection

A total of 3ml of blood was collected into EDTA venous blood collection tubes (Vacutainer , BD) and maintained at room temperature until processed within 24h.

Flow cytometry

The expression of adhesion molecules ICAM1 and β1-integrin was measured by staining whole blood samples with a FITC-conjugated monoclonal antibody mouse anti-human ICAM1 (clone 15.2 ,Chemicon) and a FITC-conjugated monoclonal antibody mouse anti-human INTEGRIN beta1 CD29 (clone TDM29, Chemicon) respectively.
The assessment of T(CD3+), T(CD4+) and T(CD8+) lymphocyte subsets was performed by staining with Tri-Test CD4-CD8-CD3 Reagent (BD) on whole blood sample.
After erythrocyte lysis with Facs Lysing Solution (BD) the samples were analysed in a flow cytometer (BD FACSCalibur) using CellQuest Pro Software.

CRP assay

The level of CRP was measured on plasma samples with an immunoturbidimetric assay (Full Range CRP, RANDOX).

Statistical Analysis

Non-parametric analysis was carried out for groups comparison by Kruskall-Wallis test. Comparison of two groups was done by Mann-Whitney U test. Significance was considered a p value <0.05.

3. Results

The distribution by etiology and location of the lesions are representative of all patients referred to the Burn Hospital during the period 1997-2011 (Fig 1, 2)

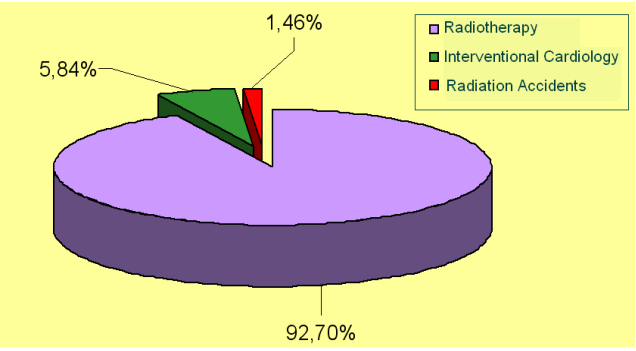


Fig 1 . Distribution by etiology of patients treated at Burns Hospital, period 1997-2011, N= 166

Late toxicity was evaluted according to the use of the RTOG/EORT
Grade1: Skin slight atrophy;pigmentation change; some hair loss
Grade 2: Patch atrophy; moderate telangiectasia; total hair loss
Grade 3: Marked atrophy; gross telangiectasia
Grade 4: Ulceration

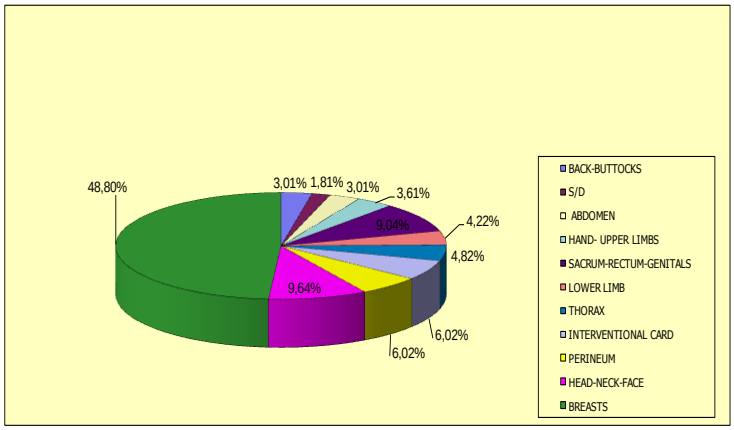
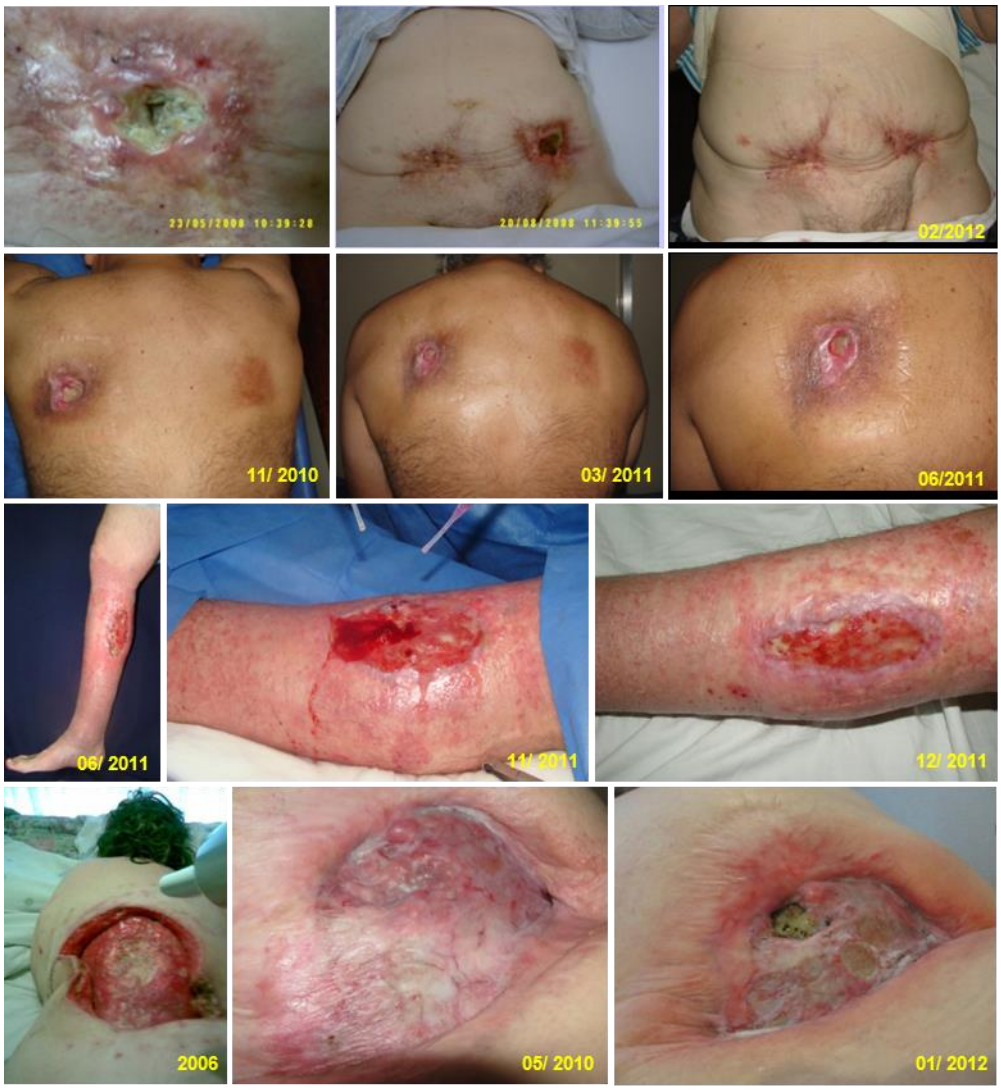


Fig 2 . Distribution by location of the lesion in patients treated at Burns Hospital, period 1997-2011, N= 166

Patient cases



Patient A
Patient who underwent Rt for ovarian cancer during mid 70s. Cyclical evolution with exacerbation crisis from 2000 up to now

Patient B
Rt for Thymoma in 1984. The patient presents at Burns Hospital in 2010 complaying from back pain and later ulceration since 2008.

Patient C
Rt for Angioma forty years ago. Ulcer recurrence over time

Patient D
Radiaton injury following interventional cardiology procedure reported as difficult, requiring prolonged duration of fluoroscopy.

CAMs analysis

The analysis of adhesion molecules expression revealed a higher expression of β1 Integrin on gated lymphocytes of Grade IV patients compared to non exposed controls (Fig 3). It was also noted a decrease in its expression value in the follow up of patients with good response to therapeutic treatment (Fig 4). There were no significant changes in the expression of ICAM1 neither lymphocytes nor granulocytes.

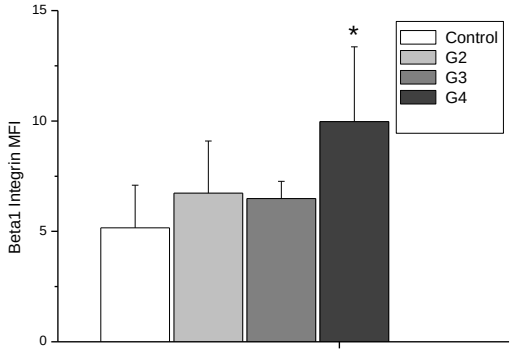


Fig 3 β1 Integrin as Mean Fluorescence Index (MFI) on gated lymphocytes of patients graded according to RTOG/EORTC System. * p< 0.05 compared to control

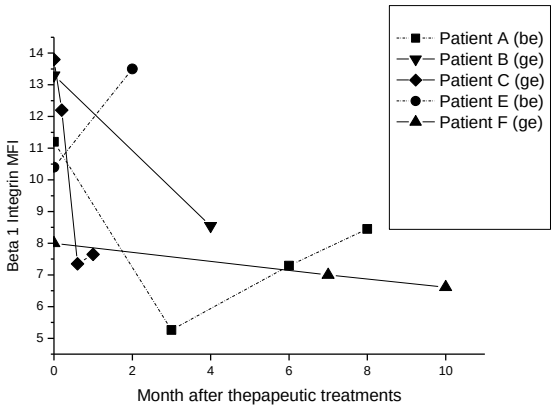


Fig 4. Changes in β1 Integrin expression on gated lymphocytes of some patients as response to medical treatment.
be: bad evolution
ge: good evolution

T lymphocytes subsets

Three-color immunofluorescence flow cytometry of lymphocyte subsets did not show significant differences in the T(CD4+) / T(CD8+) ratio among the groups, including the control one. A distortion in the frequency of thymic precursors CD4-CD8- (Double negative DN) and CD4+CD8+ (Double positive DP) in peripheral blood was observed in G4 patients (Fig 5).

C-reactive protein

The level of C Reactive Protein (CRP) , a widely used inflammatory marker, showed higher values in patients in acute phase (52,1±47.4mg/L*) and patients with late toxicity but in exacerbation crisis (13.5± 5.3mg/L*) with respect to patients with late radiation injury (1.9± 1.4mg/L). * p< 0.01

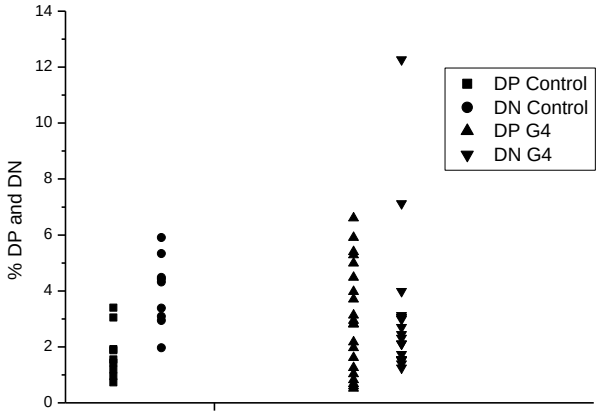


Fig 5. Percentage of Double Positive and Double Negative thymic precursors in G4 patients and control sample

4. Discussion

Scientific evidences are supporting the view that leukocyte and endothelial cell-associated CAMs play a critical role in the vascular dysfunction and tissue injury associated with a wide variety of inflammatory diseases. The coordinated recruitment of leukocytes to sites of inflammation is largely governed by the time-course and magnitude of CAMs expression.

The β1 Integrin is the major integrin expressed on resting T and B lymphocytes whereas ICAM1 mediates both lymphocyte and monocyte adhesion but its expression is regulated on endothelial cells [5].

We noted increased β1 Integrin expression on gated lymphocytes of patients that showed late cutaneous reactions graded 4 according to the RTOG / EORTC system and it had good correlation with the patient evolution . This findings differ from a previous study [1] in two affected individuals , six years after a radiation accident, that revealed elevated levels of ICAM1 and β1 Integrin on gated granulocytes.

The main effectors of the adaptive cellular immune response are CD4+ and CD8+ T cells. We have not found significant changes in the ratio of the percentage of CD4+ and CD8+ T cells, although a tendency to a decrease was observed in the G4 group of patients compared to controls.

These cells derive from precursors migrating from the bone marrow to the thymus where single positive CD4 or CD8 naive T cells are selected in a maturation process. A small proportion of circulating CD4+CD8+ (Double positive DP) or CD4-CD8- (Double negative DN) cells, representing immature T cells escaping from the thymus , has been described in humans.[6,7] Previous evidence has suggested that their frequency in blood can increase during several inflammatory disorders. The DP phenotype has been associated with high level of IL-4 production and enhanced extracellular matrix deposition by fibroblast [8].

Our data show a tendency to higher values of both, DP and DN T cells on G4 graded patients compared with control donors. This suggests a disturbance in the T-Cell homeostasis although the role played in the radiation induced damage is still largely unknown.

5. Conclusions

The present findings show that the parameters analysed, which require confirmation in a larger study , in combination with other inflammatory indicators could be used as potential follow-up markers of the chronic radio-induced inflammation process just as its response to therapeutic treatments.

6- References

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