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Use of genotoxicity, oxidative stress and inflammation blood biomarkers to predict the occurence of late cutaneous side effects after radiotherapy in Merkel cell carcinomas patients

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Despite the technical advances in radiotherapy, **toxicity to healthy tissues remains the limiting factor of treatment**. The aim of this work is to **highlight one or more blood markers whose *ex vivo* variations after irradiation (IR) could predict the occurrence of late cutaneous side effects**. For this preliminary study, we selected **patients with Merkel cell carcinoma (MCC)** because, although rare, their treatment only includes radiotherapy and the dosimetry data are accurate to the skin. **Two groups of patients were established according to the grade of late cutaneous toxicity** after adjuvant IR for MCC: (i) no or little toxicity (grade 0, 1 or 2 of the RTOG), (ii) marked toxicity (grade 3 or 4 of the RTOG). In order to try to discriminate these 2 groups, markers of interest were selected and measured on the different compartments of ***ex vivo* irradiated blood**. At the lymphocyte level, **cell cycle** and **apoptosis** (flow cytometry) and **genotoxicity** (micronuclei) were quantified. Oxidative stress was evaluated by the determination of the erythrocyte activity of the main **antioxidant enzymes** (SOD, CAT, GPx) as well as the generated **degradation products** (carbonyls and LPO in erythrocytes, secretion of 8-oxodG in plasma). The **inflammatory phenomena** were measured in the plasma by flow cytometry using LEGENDplex kits: IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1 (CCL2), IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33. Since February 2017, 18 patients treated for a MCC at Cancer Center François Baclesse (Caen, France) were included in the study. Trends, but also some significant differences in blood markers tested after *ex vivo* irradiation were observed, most often after a dose of 10 Gy. The originality of this preliminary study is based on the pool of markers used on a homogeneous population of patients. The results will then be confirmed in a prospective framework.

Materials and Methods

Two groups of patients (n=10) were constituted among the patients treated by radiotherapy for a MCC (Merkel Cell Carcinoma) at the Cancer Centre François Baclesse (Caen, France). One of these groups presented extensive skin lesions (≥ grade 3 RTOG) called « Tox ≥ 3 ». The other group presented no or few skin side effects, it was called « Tox ≤ 2 ». MCC is a rare pathology but was chosen because its treatment is only radiotherapy (no hormone therapy, no chemotherapy) and dosimetry to the skin is well defined in technical records. For the protocol, see Figure 1.

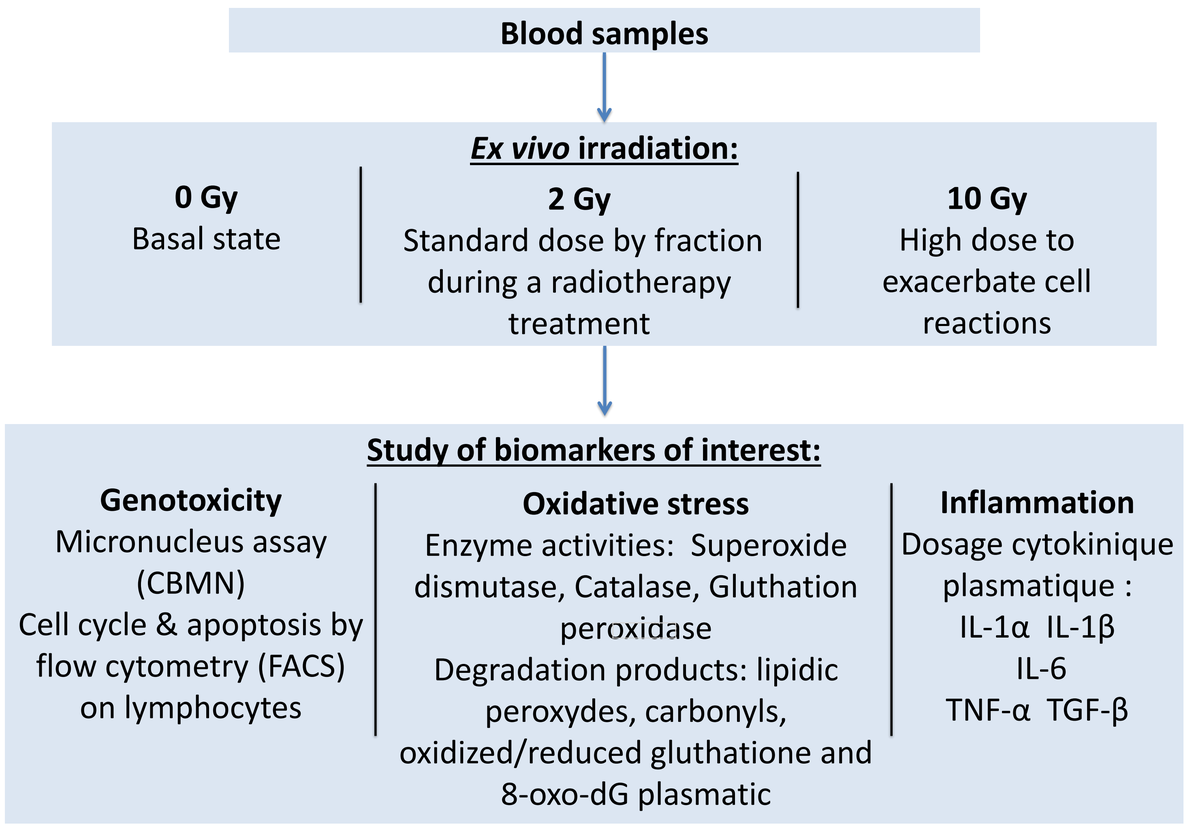


Figure 1. Experimental protocol of PAESCART study.

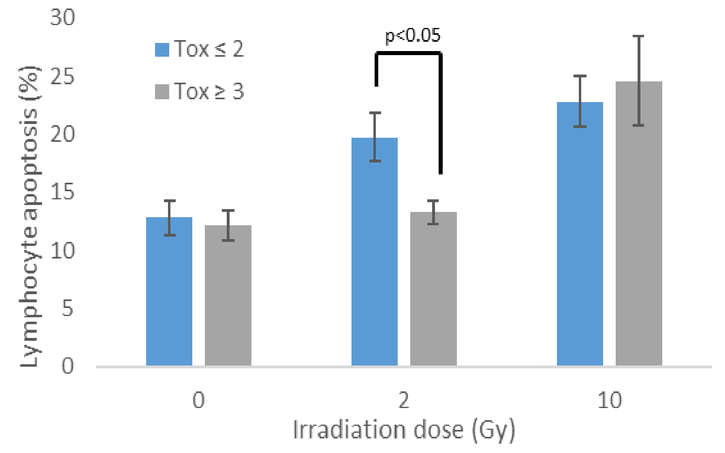


Figure 2. Lymphocyte apoptosis in both groups of patients.

Results

Eighteen patients were included since February 2017: 9 in the group « Tox ≥ 3 » (mean age = 74.4 years) and 9 in the group « Tox ≤ 2 » (mean age = 73.7 years). No significant differences were observed between patients in terms of age, received irradiation dose etc. At 2 Gy, there is a decrease in lymphocyte apoptosis (sub-G1 peak) in the group of patients « Tox ≥ 3 » compared to the group « Tox ≤ 2 » (Fig. 2). Micronucleus frequency is also decreased in the same manner but at the irradiation dose of 10 Gy (Fig. 3). At 10 Gy, there is also a decrease in SOD activity in the group « Tox ≥ 3 » (Fig. 4). In th same way, a decrease was observed in the level of reduced and oxidized glutathione (Fig. 5). There is no significant decrease but only a trend concerning IL-8 (Fig. 6) and other inflammatory cytokines, as well as degradation products as carbonyls, lipid peroxides (data not shown).

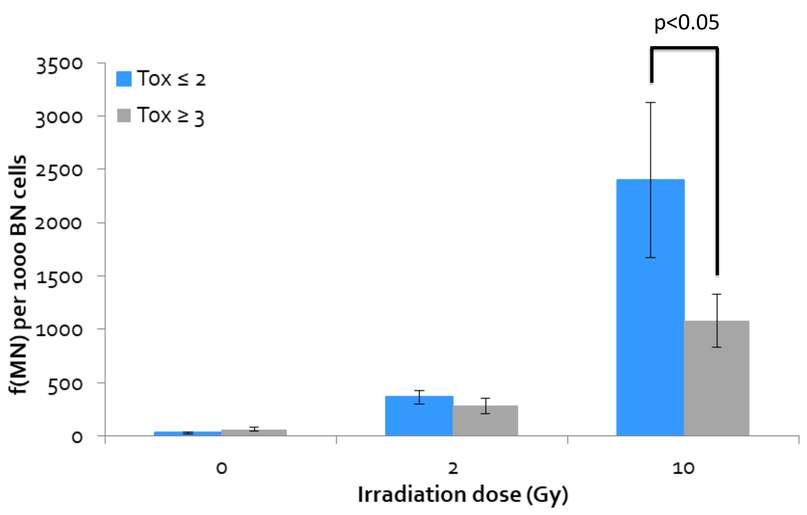


Figure 3. Micronucleus frequency per 1000 binucleated lymphocytes in both groups of patients.

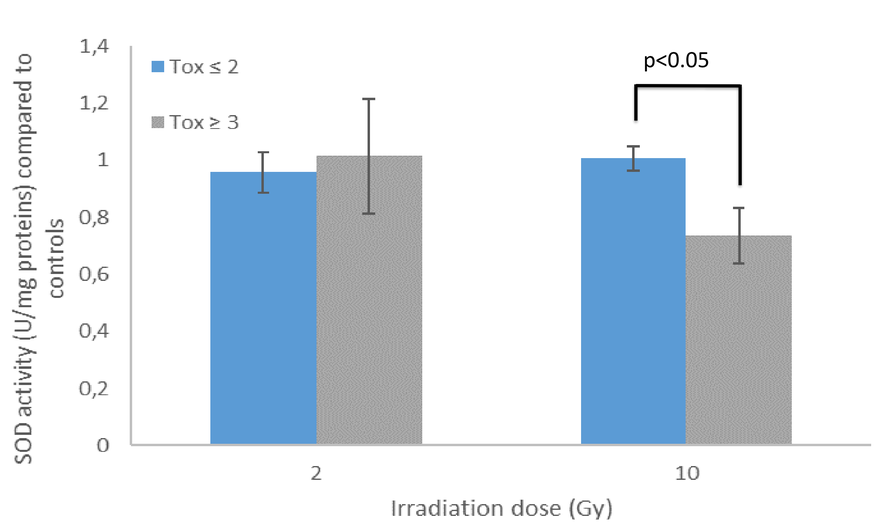


Figure 4. Superoxide dismutase activity in erythrocytes in both groups of patients. Mean value in unirradiated samples is 6.8 UI/mg of proteins.

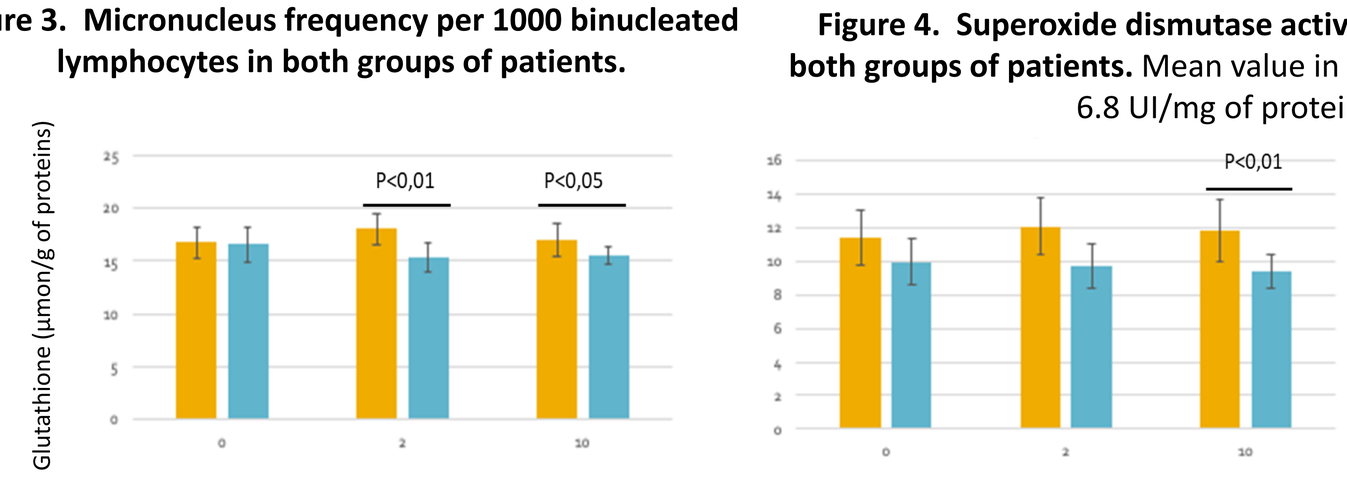


Figure 5. Reduced (left) and oxidized (right) glutathione in erythrocytes in both groups of patients.
Orange is group « Tox ≤ 2 » and blue is group « Tox ≥ 3 ».

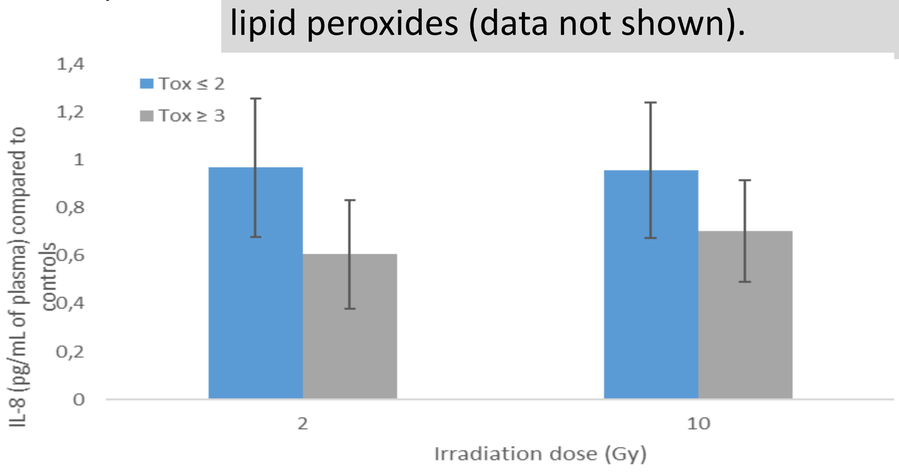


Figure 6. IL-8 concentration in plasma in both groups of patients.
Mean value in unirradiated samples is 223.4 pg/mL of plasma.

Conclusions

On the 18 patients recruited for this study, differences were observed in blood biomarkers after *ex vivo* irradiation. **Patients the most radiosensitive** seemed to present a **decrease in defense mechanisms** resulting in a **decrease in ROS detoxification**. Decrease in superoxide dismutase activity could lead to a lower production of hydrogen peroxide which could generate **less DNA damage and apoptosis**.

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