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**ALTERED APOPTOTIC PROFILES IN IRRADIATED PATIENTS WITH INCREASED TOXICITY**

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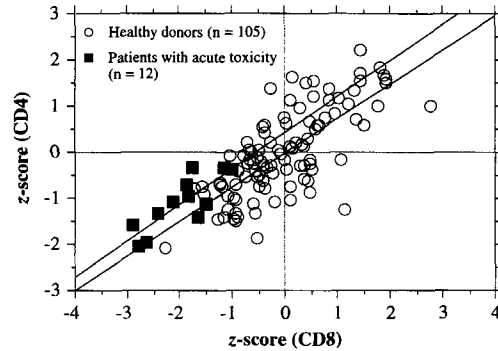
**Purpose:** Using the Leukocyte Apoptosis Assay we have previously demonstrated that mutation of the *Ataxia Telangiectasia* gene results in compromised radiation-induced apoptosis in T-lymphocytes (Ozsahin *et al*; Int J Radiat Oncol Biol Phys 1997; 38: 429–440). A retrospective study of radiation-induced apoptosis in T-lymphocytes from oncology patients, who display high acute-toxicity to radiation therapy, was performed to test for compromised response.

**Materials & Methods:** As described in Menz *et al.* (Radiat Environ Biophys 1997; 36: 175–181) briefly, 3 ml of heparinized blood was sent via express post to the PSI for subsequent examination. The blood was diluted 1:10 in RPMI medium, irradiated with 0, 2, or 8 Gy; and left to incubate for 24 or 48 h. CD4 and CD8 T-lymphocytes were then labelled using FITC-conjugated antibodies, erythrocytes were lysed, and the DNA stained with propidium iodide. Subsequently, cells were analyzed using a Becton Dickinson FACScan flow cytometer. Radiation-induced apoptosis is recognized in leukocytes as a reduced DNA content attributed to apoptosis-associated changes in chromatin structure.

Patients' data, *i.e.*, radiation-induced apoptosis in CD4 and CD8 T-lymphocytes after 2 and 8 Gy, was compared to a standard data-base of 105 healthy blood-donors aged 20 to 70 years. Apoptosis was confirmed by microscopy, electron microscopy, and by the use of commercially available apoptosis detection kits (*in situ* nick translation and Annexin V). To integrate the radiosensitivity values from CD4 and CD8 T-lymphocytes, z-score analysis was performed.

**Results:** A cohort of 12 patients aged 51–75 years who displayed high acute-toxicity was evaluated. The cohort displayed less radiation-induced apoptosis ( $-1.3 \sigma$ ) than average healthy age-matched donors. Only 1/12 displayed more apoptosis, and 9/12 were more than one standard deviation from the control mean. Although the patients all had a previous history of radiation exposure and all displayed abnormally high spontaneous levels of apoptosis, there was no indication that previous radiation history influenced subsequent radiation-induced apoptotic response.

**Conclusions:** The Leukocyte Apoptosis Assay could be used to predict individuals likely to display increased acute toxicity to radiation therapy. Validation requires a prospective study.



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**SEQUENCE-ANALYSIS OF THE ATM-GENE IN TWENTY PATIENTS WITH RTOG GRADE 3 OR 4 SEVERE ACUTE AND/OR LATE TISSUE RADIATION SIDE EFFECTS**

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**Objective:** Besides progressive neurological disorders and conjunctival telangiectasia homozygous carriers of the ataxia telangiectasia gene (ATM) show an elevated cancer predisposition and radiation sensitivity. Family studies suggest that the clinical silent heterozygous carriers of the autosomal recessive ATM may have a 3.5 to 3.8 higher risk developing cancer and may make up app. 5% of all patients with malignant diseases. In vitro studies on heterozygous lymphocytes and fibroblasts show a moderately increased cellular radiation sensitivity. This may correlate with an elevated clinical radiosensitivity of the heterozygous ATM-carriers. Therefore we analysed 20 patients of our clinic who showed severe reactions to our standard radiation treatment for heterozygosity of the ATM-gene.

**Material & Methods:** 20 patients (breast 11, rectal 2, ENT 2, prostate 1, anal 1, astrocytoma 1, Hodgkin 1) with grade 3 to 4 (RTOG) acute and/or late tissue radiation side effects were selected and gave their informed consent for genetic analysis. The patient's DNA was isolated from peripheral blood and the 66 exons of the ATM gene were amplified by PCR. Screening for larger deletions or insertions was done by agarose gel electrophoreses of the PCR-products. Then the exons were searched for mutations by a combination of single stranded conformation polymorphisms (SSCP) and automated and direct sequencing.

**Results:** We did not find significant mutations in any of the 20 patients analysed. A sequence polymorphism in one patient was found in exon 24, position 3162, a variant, which is seen in middle-european individuals. In a breast-cancer patient we found a 130 basepair (bp) insertion in intron 56. We did not find this insertion in 400 control individuals but a second carrier of this insertion was found in 125 breast cancer patients analysed.

Further bandshifts were seen in intron 25 and 26. None of the proven alterations of the ATM-gene lead to a major alteration of the gene product. **Conclusion:** The development of a so called predictive assay, which helps the clinician to define highly radiosensitive patients pretherapeutically, in order to prevent severe acute and late side effects has been one of the major goals in clinical radiology in the last few years. Because AT heterozygosity might cause an elevated radiation sensitivity we decided to analyse unusually radiosensitive patients of our clinic for the ATM-gene. Rather unexpectedly we did not find any heterozygous ATM carriers in all 20 selected, highly radiation sensitive patients. These results show that in our study-patients different genetic predispositions or other exogeneous factors may have led to the clinically observed increased radiosensitivity. The length of the ATM-gene and the variable location of the mutations found in homozygous carriers make a study on a larger cohort of patients rather difficult. Nevertheless extension of these data might be worthwhile to define the prevalence of the ATM-mutations within the group of radiotherapeutically treated patients.