The effects of computed tomography derived low doses on human peripheral blood cells

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Low dose radiation effects on the immune system: current knowledge and future research needs

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Introduction

- **Persons at risk for repeated radiation exposure (healthcare professionals, nuclear industry workers) are regularly monitored and restricted to effective doses of 100 mSv every 5 years (max. 50 mSv/year);**
- **Radiation exposure in patients who underwent medical imaging procedures is not typically monitored;**
- **Diagnostic imaging using x-rays continue to be the most common application of radiation in medicine (radiography, fluoroscopy, computed tomography, interventional radiology and bone densitometry);**
- **As the use of computed tomography (CT) has increased rapidly in the past few years, we considered that evaluations in patients who have undergone this examination can provide some valuable information on radiation risk;**
- **The aim of our study was to estimate the immediate cytogenetic effects of the exposure to low doses of irradiation on human peripheral blood cells during CT examination.**

Computed tomography (CT)

- **Inception of CT in 1970;**
- **Use has increased rapidly (7 fold for the entire population in the past decade; 15% of the total x-ray examinations; 70% of the radiation doses from medical procedures);**
- **The reasons for the increase in utilization: extremely rapid scanning; user-friendly three-dimensional software programs and multi-plannar imaging;**
- **CT involves higher radiation doses than the conventional x-ray imaging procedures (10-20 mGy vs. 0.02-10 mGy);**
- **Doses (expressed in *absorbed dose*, *organ dose* or *effective dose*) depend on a variety of factors: number of scans; tube current; tube voltage; the size of the patient; the scan pitch; the slices thickness etc.**
- **CT types: - axial CT – for head scans;**
  - helical or spiral CT – for body scans;
- **Indications: - diagnosis in sympthomatic patients;**
  - screening in asympthomatic patients.

Patients and methods

Patients: 13 untreated subjects, with different types of malignancies, subjected to CT, divided into two groups:

- 6 were given cranial CT (CRN-CT), Pace (General Electrics, Japan) - mean absorbed dose of 17.5 mGy;
- 7 thorax-abdomen-pelvis CT (TAP-CT), Aura (Philips, Netherlands) - mean absorbed dose of 43.85 mGy ;

- blood samples were collected before and immediately after CT;
- determinations were assessed on whole blood or peripheral blood mononuclear cells (PBMCs) isolated from whole blood in density gradient.

Methods:

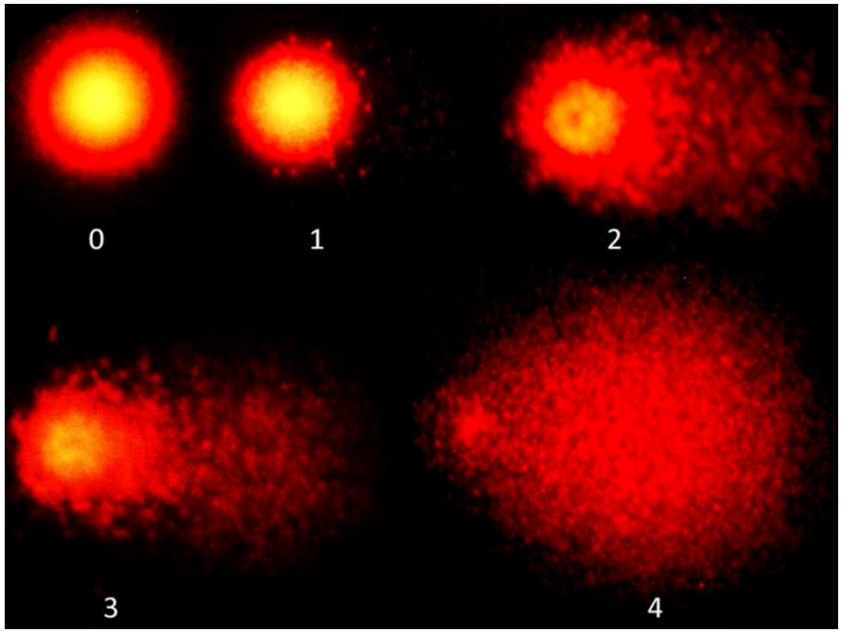
- the basal DNA lesions and those induced by irradiation were determined in fresh blood cells by Single Cell Gel Electrophoresis or Comet assay (CA);
- the potential chromosomal aberrations, induced by ionizing radiations were evaluated from cryopreserved PBMCs with Cytokinesis Blocked Micronucleus assay (CBMA);
- statistical processing of the experimental data was accomplished using GraphPad Prism software program, version 5.0 (GraphPad, San Diego, Ca, USA) and Excel. Statistical comparison between groups was made by *t* Student Test ($p<0.05$ statistical significance).

Single Cell Gel Electrophoresis or Comet assay (CA)

Is a highly sensitive method for the assessment of DNA damage formation and repair (*Tice et al., 2000*), briefly:

- **leukocytes from whole blood were embedded in low melting agarose;**
- **cells were layered onto slides precoated with normal melting agarose;**
- **the "cell-slides" were immersed in lyses solution to remove all non-nucleic material;**
- **electrophoresis was conducted in alkaline buffer (pH 13.2) at a field strength of 25V/300mA;**
- **slides were neutralized, fixed with ethanol, stained with Ethidium bromide and incubated in the dark;**
- **for the visualization of DNA damage an epifluorescent microscope was used (excitation filter: 510-560 nm, barrier filter: 590 nm) and 200 cells were analyzed per sample.**

Representations of comet images and classes and parameters calculated by CA: *lesion score (LS)* and *tail factor (TF)*



- **SL [UR]= $\frac{A\cdot 0+B\cdot 1+C\cdot 2+D\cdot 3+E\cdot 4}{5}$**

A,B,C,D,E – number of cells in comet classes 0,1,2,3,4

- **TF [%] = (A·FA+ B·FB+ C·FC+ D·FD+ E·FE)/ Total number of cells**

A,B,C,D,E – number of cells in comet classes 0,1,2,3,4

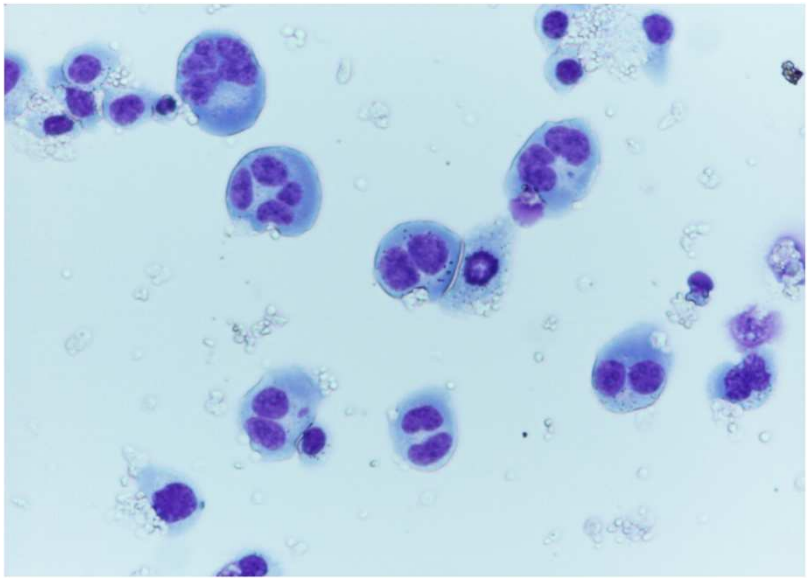
FA-FE – mean proportion of DNA in the comet tail

The Cytokinesis Block MicroNucleus assay (CBMA)

Is one of the most used methods for assessing unstable chromosomal aberrations, such as acentric chromosomal fragments, which are visible in interphase as extra nuclear corpuscles named micronuclei (*Fenech, 2000*), briefly:

- **PBMCs isolated before and after CT were treated with Phytohemagglutinin M (PHA) to stimulate cell division and to ensure maximum yield of binucleated cells (20 µg PHA/ml cell culture);**
- **Cytochalasin B (Cyt B) was added to block cytokinesis process after 44 hours of incubation (6 µg Cyt B/ml cell culture);**
- **cells were harvested by cytocentrifugation at the end of the 28 hours incubation with Cyt B;**
- **the cells were stained with Giemsa 10% and examined at 1000x magnification.**

Representations of binucleated cells and micronuclei and mathematical values obtained by CBMA

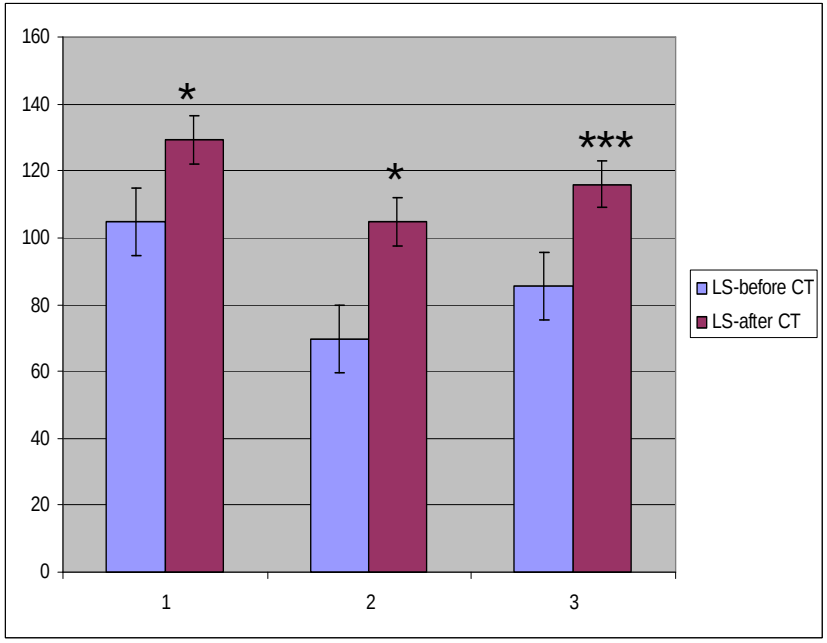


Giemsa stain, 1000x

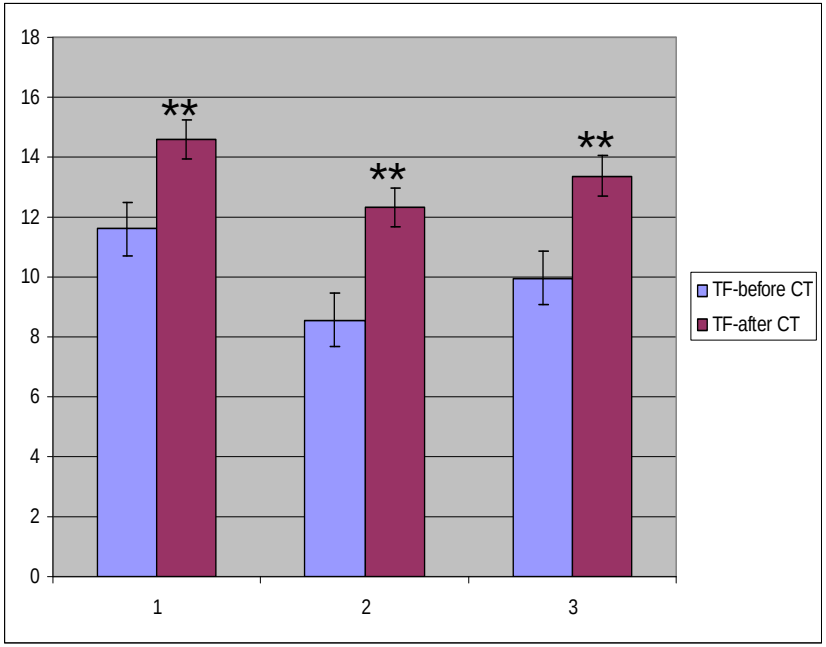
- Percentage of binucleated cells with micronuclei (%Bi+Mni)
- Number of micronuclei in 1000 binucleated cell (Nr.Mni/1000Bi)

Mean values of the CA determinations expressed by: *lesion score (LS)* and *tail factor (TF)* in patients subjected to CT

LS [UR]



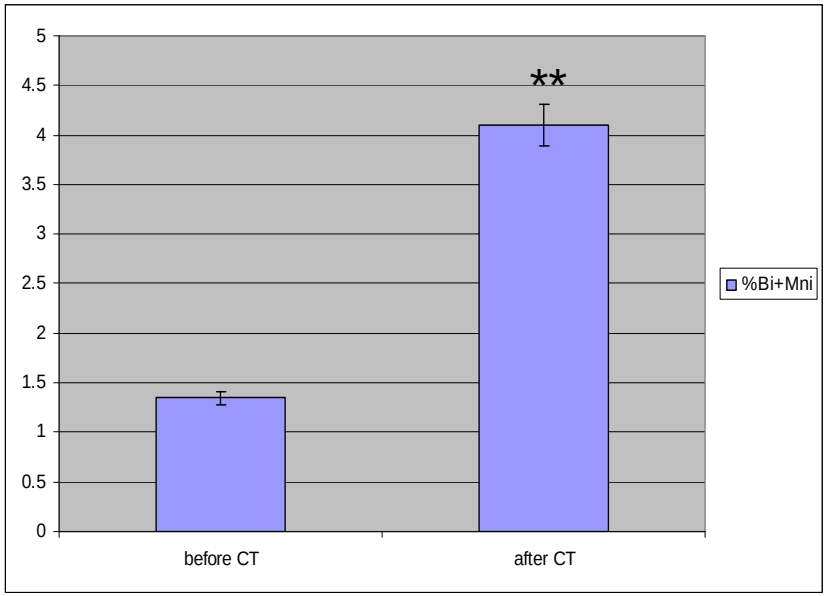
TF [UR]



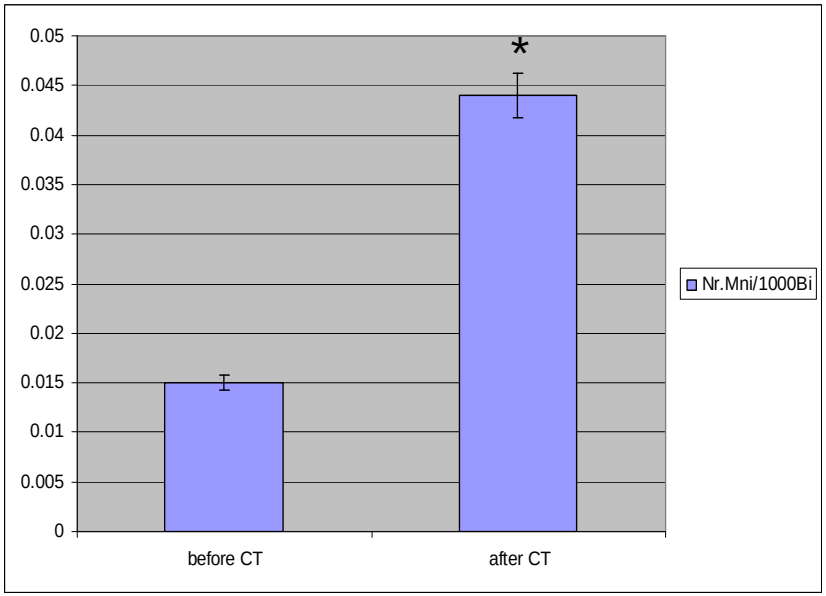
1-CRN; 2-TAP; 3-global evaluation

Mean values of the CBMA determinations evaluated by: *%Bi+Mni* and *Nr.Mni/1000Bi* in patients subjected to CT

%Bi+Mni



Nr.Mni/1000Bi



Global evaluation

Conclusions

- **Low-doses of irradiations, provided by CT, can cause detectable cytogenetic modifications in human blood cells, immediately after exposure, which consists of DNA lesions and chromosomal aberrations.**
- **There are noticeable interindividual differences between basal levels of DNA lesions and chromosomal aberrations.**
- **The DNA lesions after CT were higher in the cranial CT group.**
- **Since, the risks of radiation are cumulative, it is essential to radiologists to reduce to the minimum the radiation dose delivered by each CT scan administered.**
- **The data presented in this work are the results of a preliminary study of the low dose radiation effects in human blood cells.**
- **For a more accurate evaluation it is recommendable to increase the number of study cases.**