Evaluation of individual *in vitro* radiation response in PBMC samples of H&N patients correlated with severity of side effects following radiotherapy.

Mihaela TEMELIE (1), Patricia MEDESAN (1), Mihaela TUDOR (1), Daniela STROE (2), Diana SAVU (1).

(1) National Institute of Physics and Nuclear Engineering Horia Hulubei, Magurele, Romania.

(2) Clinical Hospital Coltea, Bucharest, Romania.

8th International Conference on Radiation in Various Fields of Research July 20–24, 2020

Introduction

Radiotherapy

- primary treatment options for Head and Neck (H&N) tumors together with surgery.

- may induce mild to very severe side effects ; can profoundly affect life quality or lead to long term damage.

The possibility to develop personalized treatment plans based on individual response to radiation would represent a major improve in cancer patients life expectancy and life quality.

AIM: correlation of *in vitro* radiation response parameters of PBMC isolated from head and neck cancer patients with severity of side effects following radiotherapy.

Materials and methods

Donors: H&N cancer patients undergoing radiotherapy at Radiotherapy Department, Coltea Clinical Hospital, Bucharest, Romania during August 2019-March 2020. Selection criteria included absence of prior exposure to genotoxic factors such as chemotherapeutic treatment.

Blood collected by venipuncture before radiotherapy and following 20 fractions of 2 Gy.

Anticoagulant – EDTA / heparin.

In vivo observations: radiotherapy side effects were determined using European Organization for Research and Treatment of Cancer (EORTC) criteria.

Radiotoxicity was determined by the following criteria: skin morphology, mucous membrane, eyes, salivary glands, pharynx , esophagus and larynx. Additionally, blood test were performed to determine level of hemoglobin, neutrophil, total white blood cells, blood platelets and haematocrit.

Deviations from normal parameters were registered by a score of 0-5, 0 being atributed to patients that presented no changes and 5 the most severe effects.

Materials and methods

In vitro evaluation:

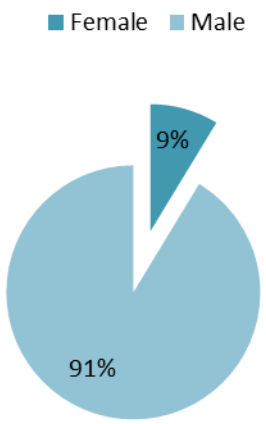
- Plasma and PBMC were isolated from blood samples.
- PBMC were exposed *ex vivo* to 0, 0.5 and 2 Gy of X-ray radiation (Linear accelerator Primus Mevatron, Siemens).
- DNA damages were evaluated by cytokinesis block micronucleus assay (at ~72 hours post irradiation) and gamma-H2AX foci measurements by manual scoring using a fluorescence microscopy (at 1 and 24 hours post irradiation).
- Cytokine levels (TNF alpha; IL-1beta; IL-6) were determined before and following 20 fractions of radiotherapy using ELISA kits.

RESULTS – Cohort description and sample distribution

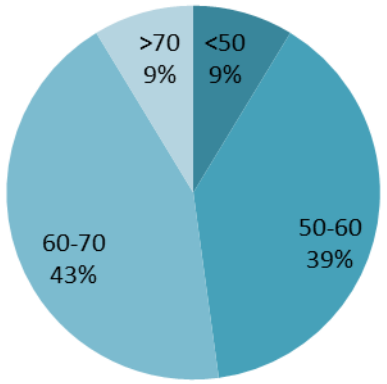
Sample	Age	Cancer type	Sex
ORL1	66	nasopharyngeal cancer	M
ORL2	59	nasopharyngeal cancer	M
ORL3	52	pharynx and larynx cancer	M
ORL6	71	tongue cancer	M
ORL4	51	nasopharyngeal cancer	M
ORL5	52	nasopharyngeal cancer	M
ORL7	59	oropharyngeal cancer	M
ORL8	62	pharynx and larynx cancer	M
ORL9	69	tongue cancer	M
ORL10	40	nasopharyngeal cancer	M
ORL11	63	oropharyngeal cancer	M
ORL13	65	tongue cancer	M
ORl14	63	larynx cancer	M
ORL17	62	oropharyngeal cancer	M
ORL15	64	tongue cancer	M
ORL16	65	laryngeal cancer	M
ORL18	46	tonsil cancer	M
ORL20	55	pelvilingual cancers	F
ORL19	58	lymphatic metastases	M
ORL21	67	oropharyngeal cancer	M
ORL22	58	pharynx and larynx cancer	M
ORL23	74	tonsil cancer	F
ORL24	56	pharynx and larynx cancer	M

- Patients included in the study were diagnosed with various types of H&N cancer;
- Most patients (91%) were male.
- Gaussian age distribution with a mean at ~60 years.

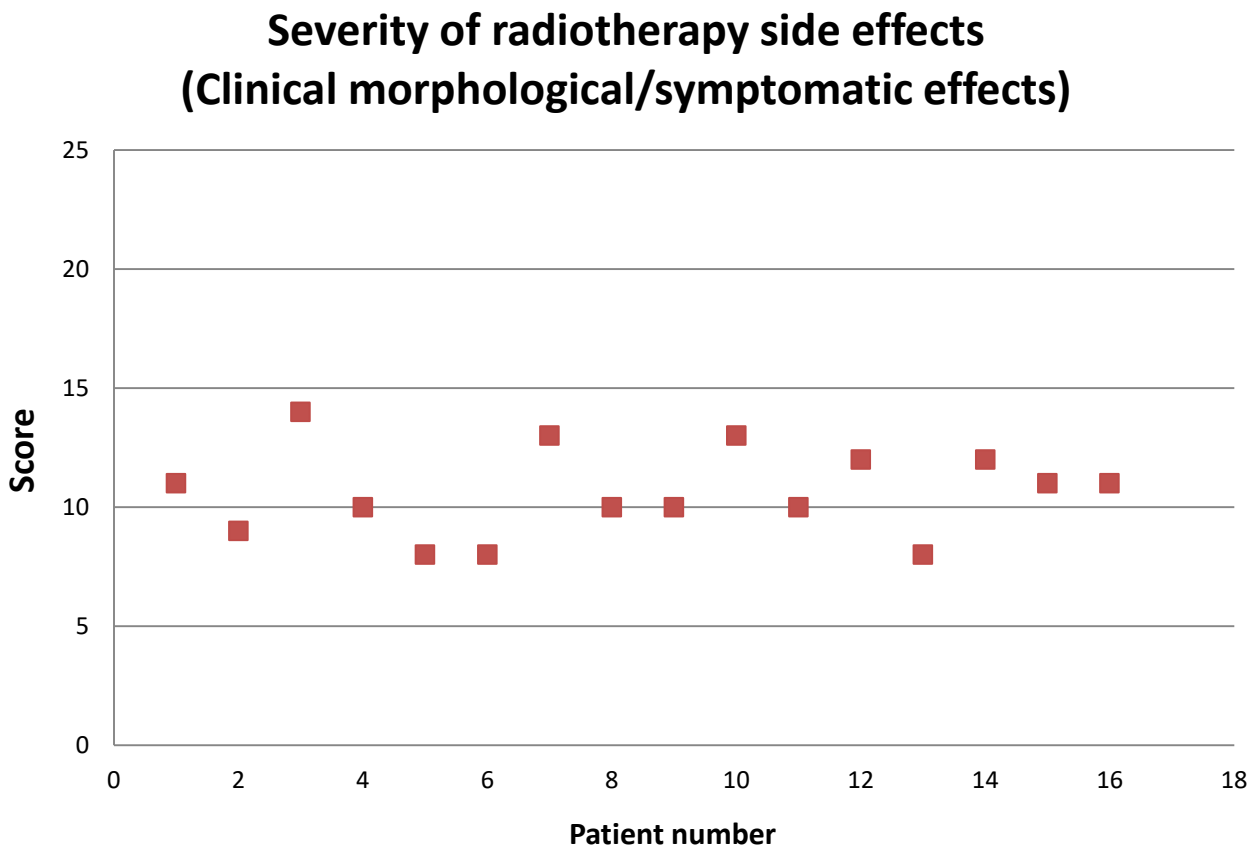
Samples distribution by sex



Samples distribution by age



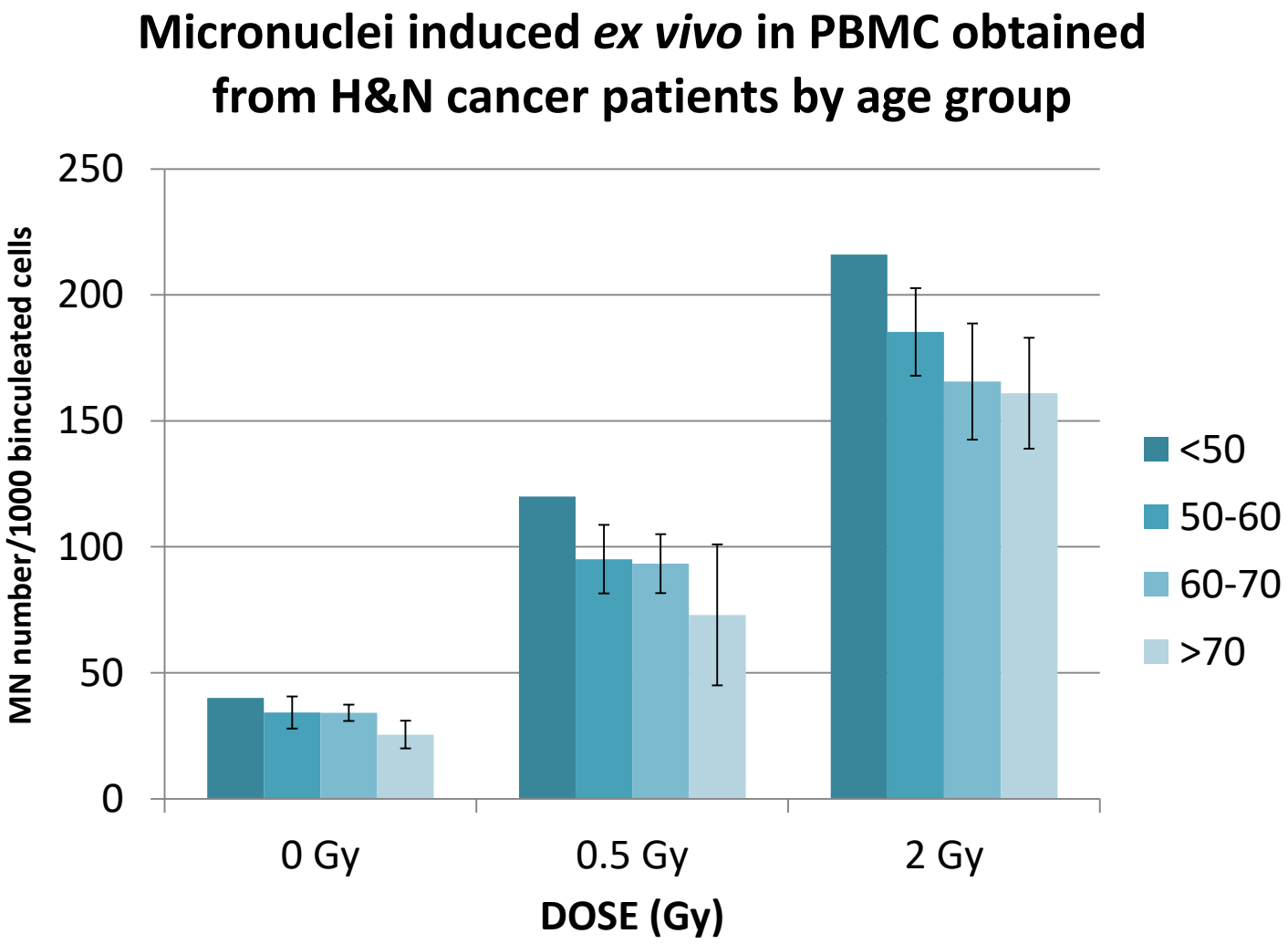
RESULTS – Severity of radiotherapy acute side effects following 20 fractions of treatment of 2 Gy



- All the subjects presented side effects with average severity.
- None of the patients presents extreme radiosensitivity/ radioresistance.
- Evaluation of haematology tests revealed mild changes in hemoglobin and haematocrit on few of the subjects.

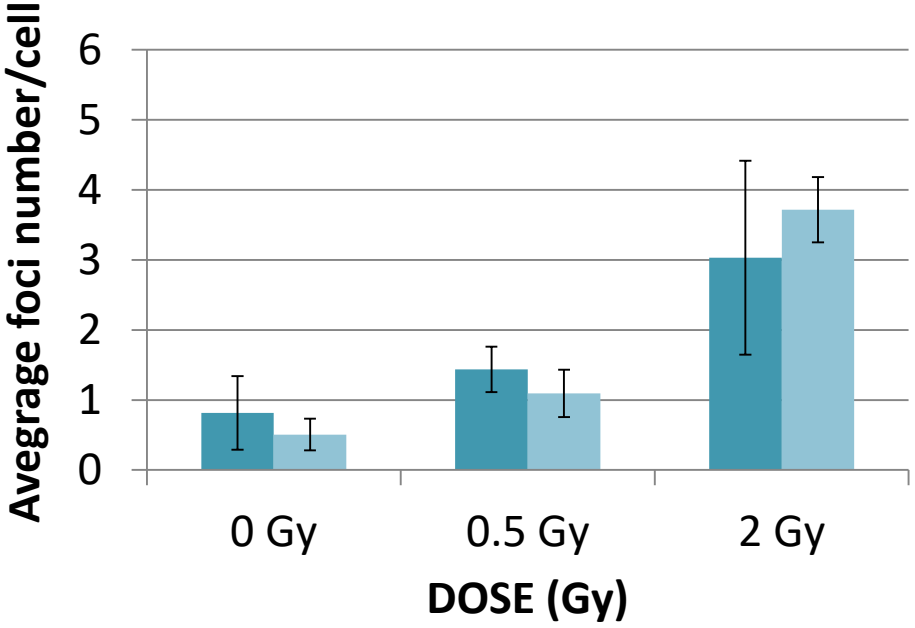
Clinical observation of skin, mucous, salivary glands, pharynx/esophagus and larynx changes following 20 fractions of radiotherapy were registered on a chart, using a scale from 0-5. 0 correspond to no change from baseline and 5 severe detrimentally effects (eg. severe ulceration, hemorrhage, necrosis, obstruction). The score obtained at each of the 5 categories were summed, obtained a final score that can theoretically range from 0 to 25.

RESULTS – Radiosensitivity - *in vitro* study – DNA damage response

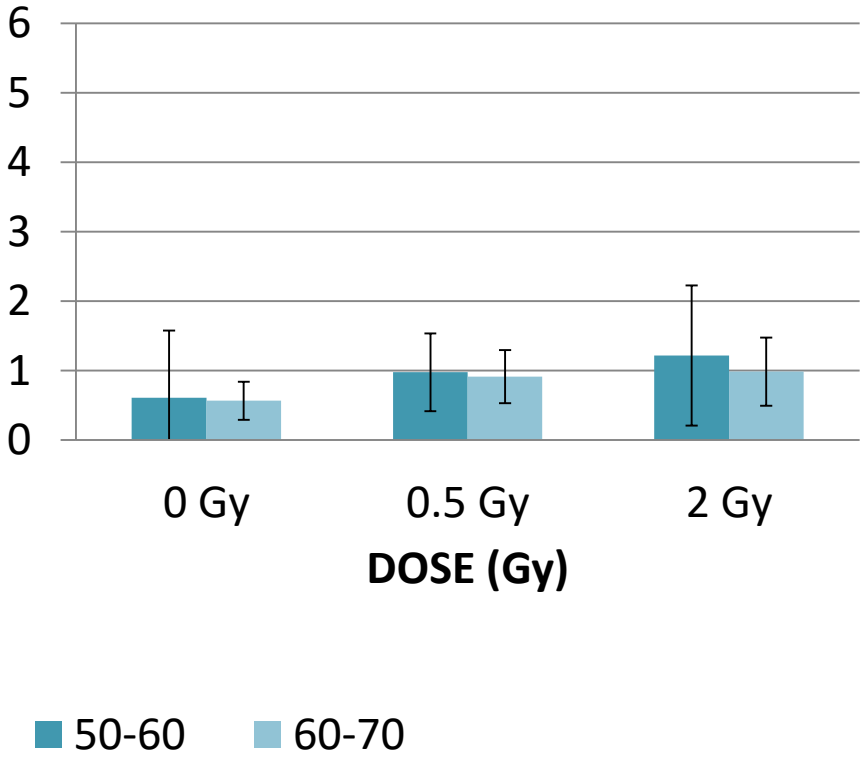


RESULTS – Radiosensitivity - *in vitro* study – DNA damage response

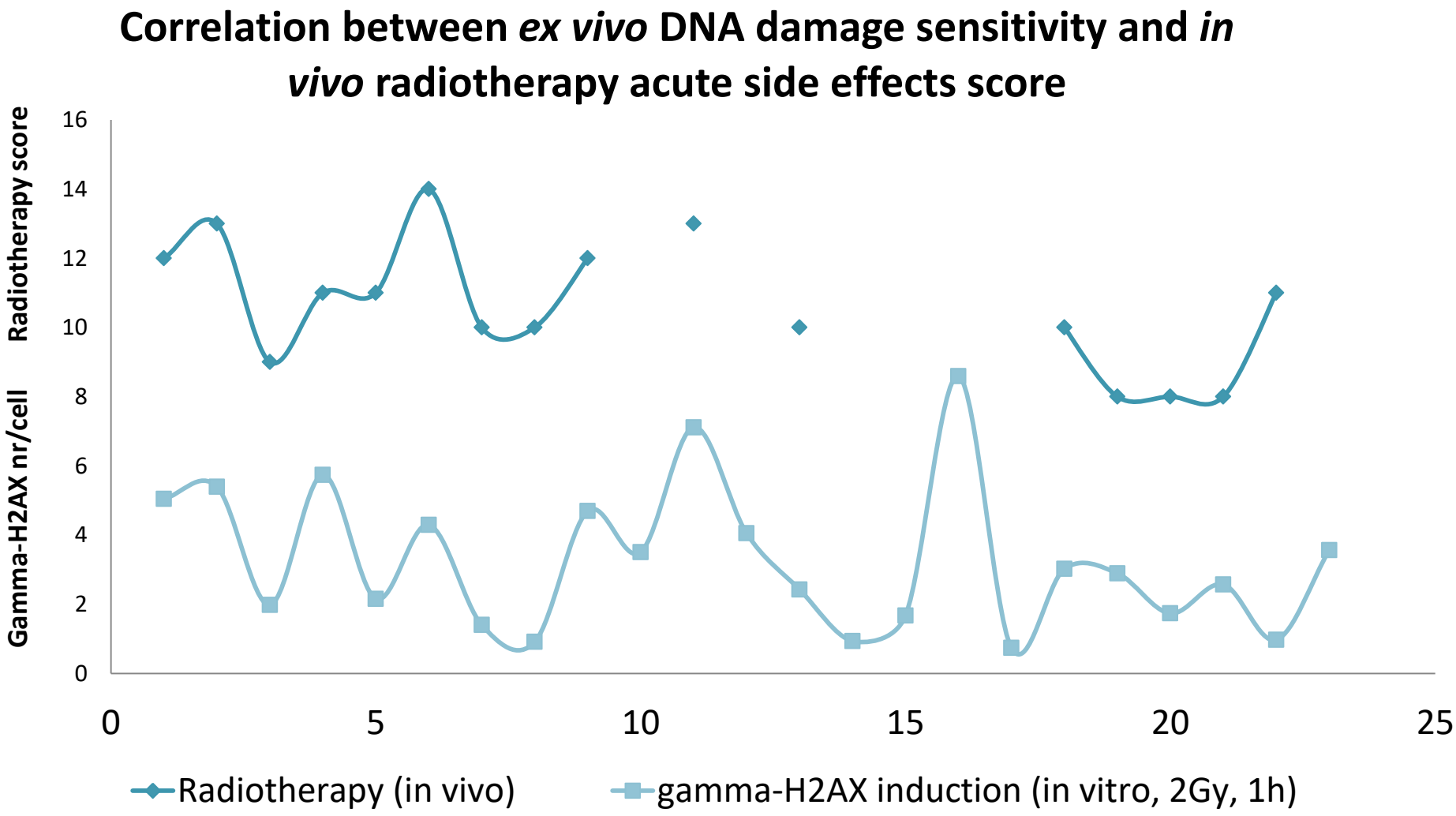
Gamma-H2AX foci induced *ex vivo* in PBMC obtained from H&N cancer patients by age group (1h post-irradiation)



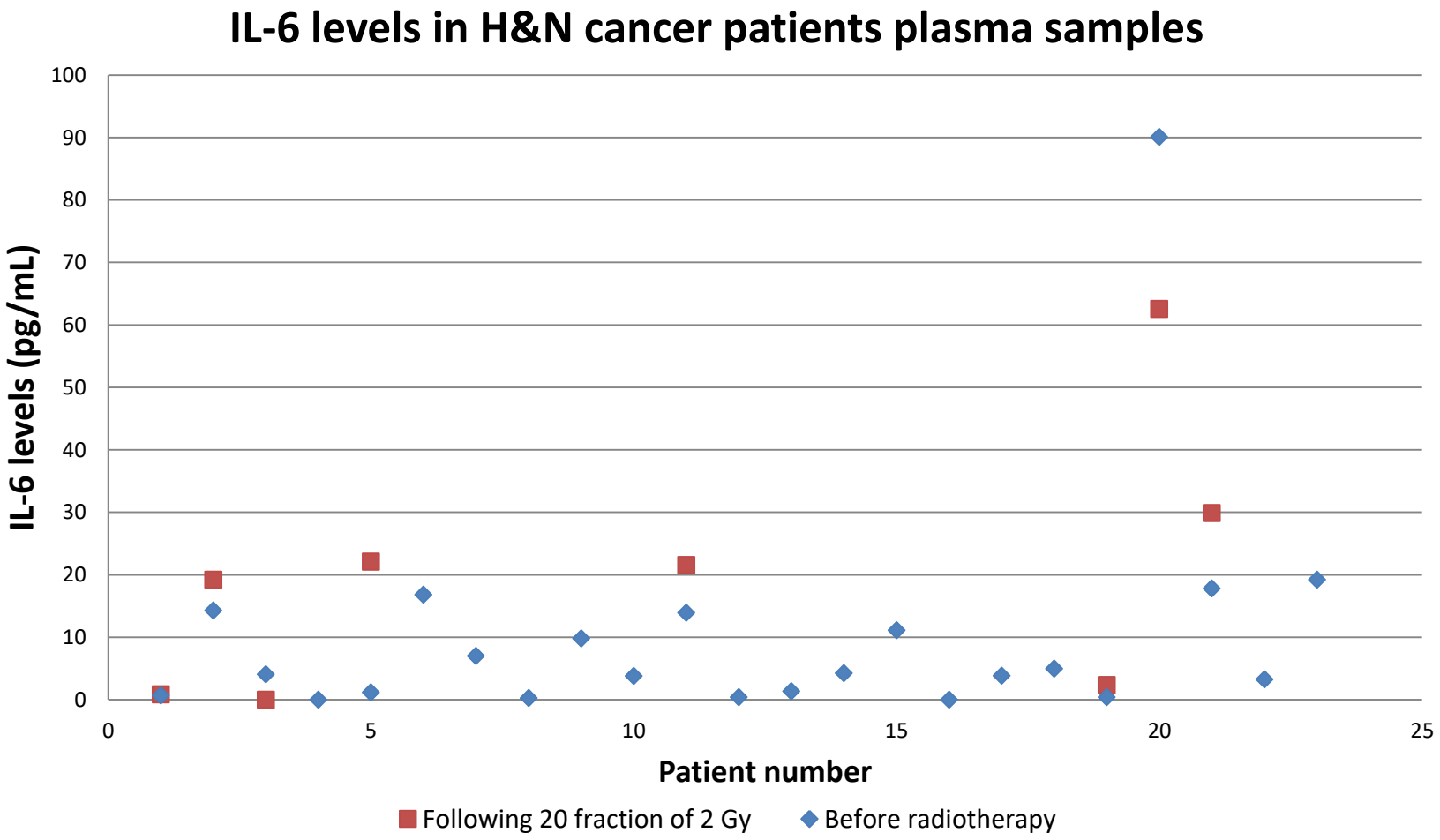
Gamma-H2AX foci induced *ex vivo* in PBMC obtained from H&N cancer patients by age group (24h post-irradiation)



RESULTS – *In vivo – in vitro* radiosensitivity correlation



RESULTS – Radiosensitivity - *in vitro* study – Cytokine measurements



- IL-1 beta measurements lead to non-detectable levels of cytokine in all samples both prior radiotherapy and following 20 fractions.
- IL-6 measurements in control sample showed non-detectable levels.

CONCLUSION

- Most H&N cancer patients undergoing radiotherapy during the study at Coltea Clinical Hospital and matching the study criteria were male (91%) with a mean age ~60 years.

- Radiotherapy side effects manifested by the subjects were moderate. None of the patients presented exacerbated or absent side effects.

- DNA damage markers – micronuclei yield and gamma-H2AX foci number revealed similar degree of sensibility to *in vitro* radiation exposure in different age groups. DNA-damage repair was similar as well.

- Inter-individual comparison of *in vitro* radiation sensibility showed great variation in the levels of radioinduced gamma-H2AX foci and repair kinetics. The variation cannot be directly correlated with the intensity of the *in vivo* acute side effects.

- IL-1 beta showed non-detectable levels in all samples (before and following 20 fractions of radiotherapy). IL-6 measurements showed non-detectable levels in control samples, and variable levels in cancer patients – from non-detectable to 90 pg/mL. Following radiotherapy, the levels of IL-6 presented a slight increase.

PERSPECTIVE

The study will lead to the cryopreservation of a large number of samples:

- Plasma obtained from blood collected prior and following 20 fraction of radiotherapy.
- PBMC cells collected at the same time.
- Cell pellet for molecular analysis (PCR/western blot) obtained from PBMC isolated before treatment and *ex vivo* exposed to 0 or 2 Gy of radiation.

The study will continue by analysis of the samples through:

- Cytokine measurements – TNF alpha;

- Microarray test on selected samples for investigating the DNA damage response pathway.

- Quantitative measurements of molecular markers of DNA damage and apoptosis.

Thank you for your attention!