Assessment of the absorbed dose: of ionizing radiation , comparison of biomarkers sampled 3 h or 24 h after exposure

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وزارة البحث العلمي

أكاديمية البحث العلمي والتكنولوجيا

*What to do if a **radiation accident** occurs!*

Imagine a scenario where a large-scale nuclear or radioactive emergency strikes, affecting countless individuals. In such critical situations, swift and accurate determination of individual absorbed doses becomes paramount to effectively allocate medical resources and provide timely interventions. However, conventional cytogenetic biomarkers and existing multiparametric software solutions fall short, typically requiring 24-50 hours to provide crucial dose information.

- Recently, a groundbreaking Italian-Egyptian collaborative study, funded by NATO SPS (G4815) and STDF PROJECT ID 25871 for 24 hrs samples led by PI **Noha Awad** and Co-PI **Anna Giovanetti** , introduced a novel panel of early biomarkers capable of measuring radiation doses just hours after exposure. This panel, validated with results from the reference biomarker micronuclei (MN) count assay, includes a comprehensive set of biomarkers such as the count of blood cells (CBC), DNA breaks (assessed by Comet assay), amylase (AML), FMS-like tyrosine kinase 3 ligand (FLT3-L), citrulline, and inflammation cytokines like IL1B, IL6, and IL 8, as well as zinc and copper concentrations.

- This innovative approach was tested on blood samples drawn from patients before and 3 hours after radiotherapy at the Istituti Fisioterapici Ospitalieri—Istituto Nazionale Tumori "Regina Elena" (IRE-IFO) in Italy and at Alexandria University Hospital in Egypt. Notably, five biomarkers—CBC, DNA breaks, IL6, zinc, and copper concentrations—were found to independently correlate with the dose delivered to a partial-body area.

- The decision to sample patients at the three-hour mark was strategic, balancing the need for rapid sampling during emergencies with ethical considerations regarding patients' psycho-physical status. However, the chosen biomarkers operate through distinct biological mechanisms, exhibiting non-overlapping kinetics and peaks of expression. Thus, the inclusion of a 24-hour sampling significantly enhances the reliability of identifying a suitable tool for radiation/nuclear (R/N) emergencies.

At Alexandria University Hospital, a third sample was obtained from patients at their homes, 24 hours after radiotherapy, offering a valuable comparison between biomarker values at various time points. In this presentation, we will delve into the detailed analysis of biomarker values in blood samples collected before, three hours, and 24 hours after radiation exposure, showcasing the potential of these early biomarkers in revolutionizing the rapid assessment of radiation exposure in emergency settings.

How we apply our study

- **Patient enrolment criteria and irradiation**

1. over 18 years of age
2. scheduled for a first dose of either 2Gy, 3 Gy or ≥5Gy
3. not currently undergoing chemotherapy; without haematological malignancy or other blood or metabolic diseases.

Study Enrollment

- 70 patients enrolled from Istituti Fisioterapici Ospitalieri—Istituto Nazionale Tumori “Regina Elena” (IRE-IFO) in Italy and Alexandria University Hospital in Egypt.
- Dose groups: 50 patients exposed to 2 Gy, 15 to about 3 Gy, and 5 to ≥ 5 Gy.

Blood Sample Collection and Analysis

- Samples collected before and 3 hours after radiotherapy using BD Vacutainer®.
- Biomarkers analyzed: CBC, DNA breaks (Comet assay), amylase, FLT3-L, citrulline, IL1B, IL6, IL8, zinc, copper.
- Reference MN count assay performed for validation.

COMET Assay

1

Cells mixed with low melting agarose at 37°C (LM Agarose)

2

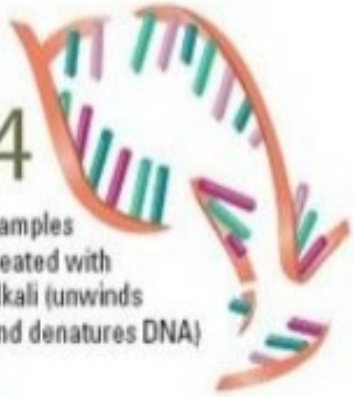
Immobilize cells on CometSlide™

3

Treat cells with Lysis Solution (removes membranes and histones from the DNA)

4

Samples treated with alkali (unwinds and denatures DNA)



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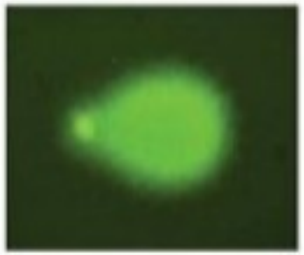
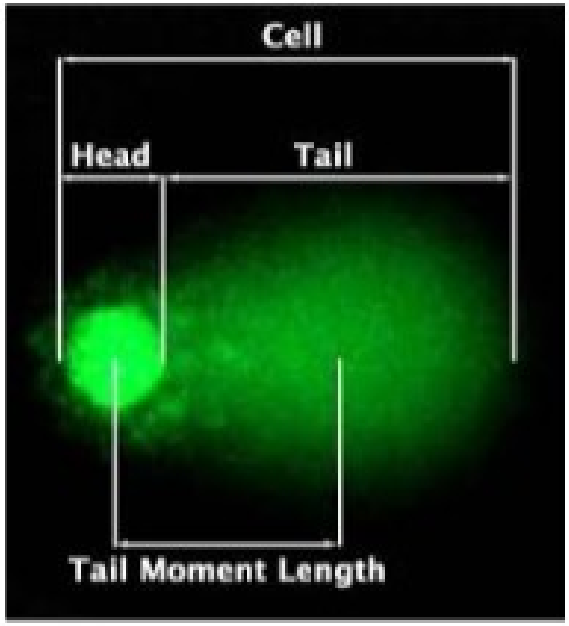
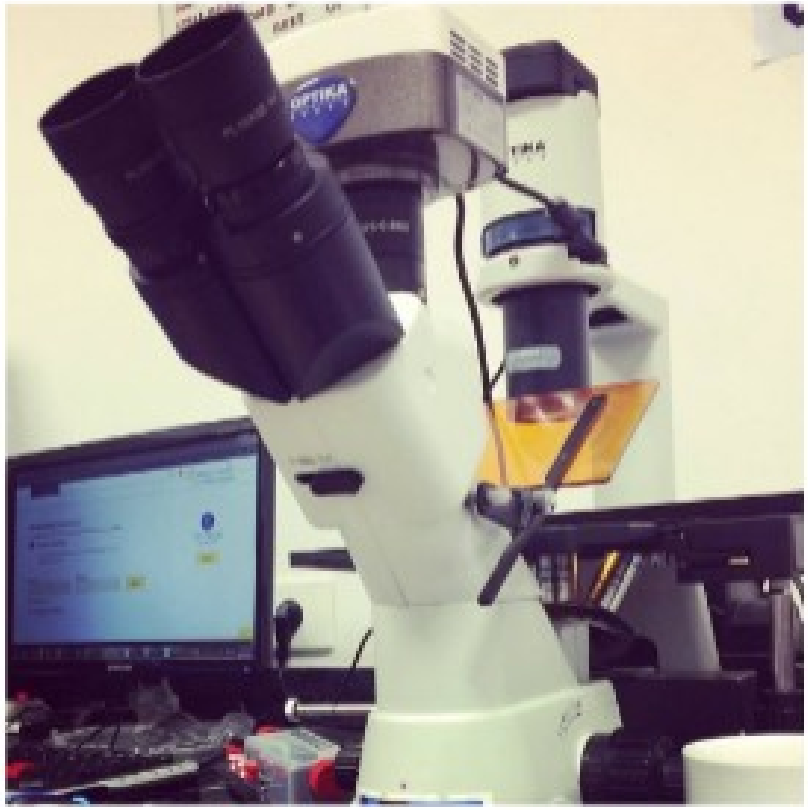
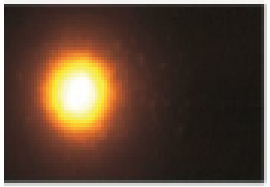


Samples stained with intercalating dye and visualized by epifluorescence microscopy following alkaline electrophoresis, which reveals DNA breaks

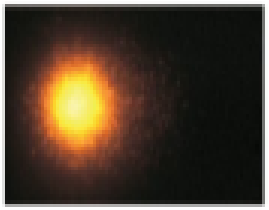
COMET Results



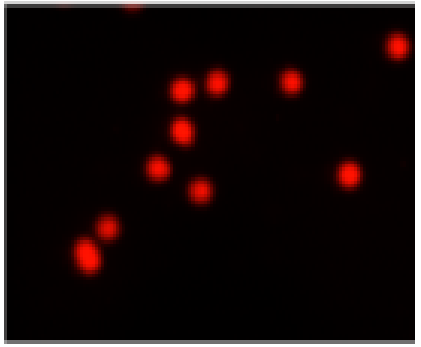


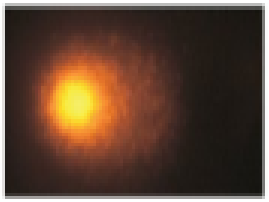


Type 1: Comet without tail where the genetic material remain inside the nucleus.: no genetic damage

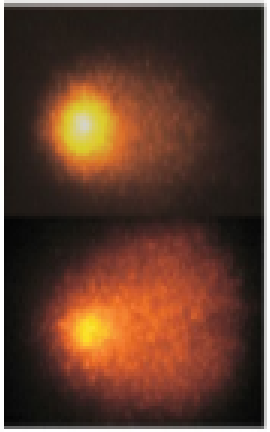


Type 2: Cell with a small tail, little migration of fragments of DNA: light genetic damage.



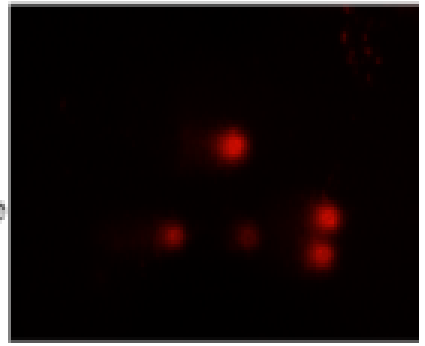


Type 3: Cell with tail with evident migration due to a greater damage.

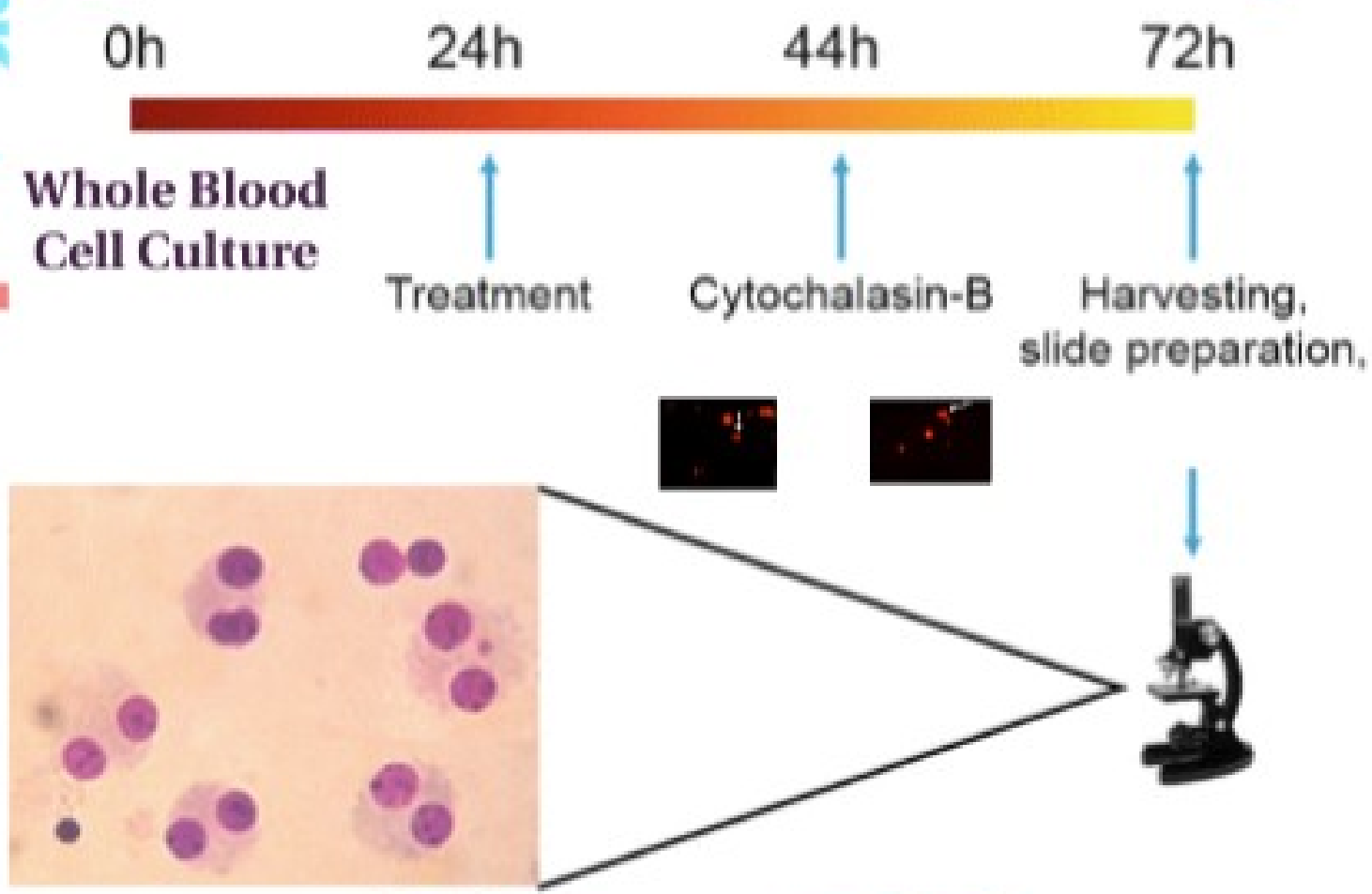


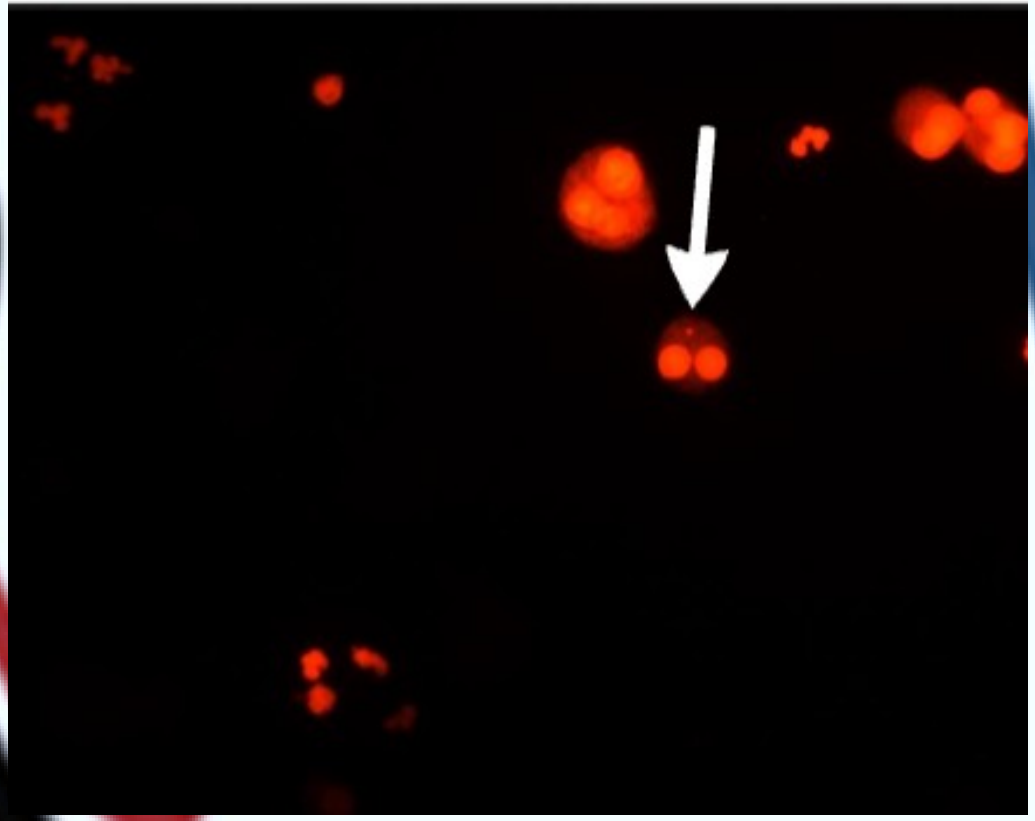
Type 4: Cell with definite tail with a consistent amount of fragments.

Type 5: Almost all the DNA is present in the tail: severe genetic damage



Micronucleus Assay





Methodology

- Cell blood counts: EDTA blood analyzed with Beckman Coulter DXH800.
- α-Amylase test: Serum samples analyzed with a colorimetric enzymatic assay.
- MN count assay: Whole blood cultured and analyzed for MN count.
- DNA breaks (Comet assay): Whole blood analyzed for DNA breaks.
- Inflammatory cytokines: Assessed on sera using the BDTM Cytometric Bead Array.
- FLT3-L concentration: Measured with an ELISA kit.
- Zinc and copper concentrations: Determined using microplate assay kits.

Patient Dosimetry

- Integral dose calculated based on DICOM data using specific formulae.
- Treatment plans generated and delivered using Eclipse treatment planning system and Clinac 2100/CD at IRE-IFO, and Peacock treatment planning system and Elekta Synergy linac at Alexandria University.

Data Analysis

- Baseline control values and percentage variations of biomarkers determined.
- Pearson correlation tests conducted to assess relationships between radiation dose and biomarkers.
- Univariate and multivariate linear regression analyses used to identify factors relevant to absorbed dose.
- ROC analysis used to calculate AUC for identifying dose group of irradiated subjects.

Conclusion

- The study validates a novel panel of early biomarkers for rapid assessment of radiation exposure.
- These biomarkers offer a potential solution for timely allocation of medical resources in nuclear or radioactive emergencies.

Future Directions

Further validation and refinement of the biomarker panel.

Integration into emergency response protocols for nuclear or radioactive incidents.

Q&A Session