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Rapid Detection of Platelet Alterations in Radiation and Combined Injury Using Flow Cytometry

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Introduction

Radiation exposure and radiation combined injury (RCI) represent major military and civilian mass-casualty threats. Platelet dysfunction contributes to trauma-induced coagulopathy and adverse clinical outcomes following these injuries, yet rapid diagnostic approaches remain limited. Flow cytometry provides high-throughput assessment of platelet morphology and activation. We developed a simple Flow Cytometry Algorithm (FCA) to rapidly detect injury-associated platelet alterations. This label-free approaches relies on two key light-scattering parameters to differentiate distinct cell populations:

- Forward scatter (FSC): Measures light scattered in the forward direction and is commonly associated with cell size.
- Side scatter (SSC): Measures light scattered at approximately 90◦ to the laser beam and is associated with cellular complexity and granularity.

Hypothesis

We hypothesized that whole-body irradiation (WB) and radiation combined with polytrauma (CI) induce measurable alterations in platelet morphology and complexity that can be rapidly quantified by flow cytometric scatter analysis. We further hypothesized that a flow cytometry Algorithm (FCA), derived from platelet FSC and SSC parameters, would correlate with injury severity.

Methods

Study Workflow

Animal Groups

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Peripheral Blood Collection (Day 14 Post-Injury)

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Platelet Identification (FSC/SSC Gating)

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FSC & SSC Parameter Extraction

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FCA Calculation
FCA = FSC × SSC

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Statistical Analysis (One-way ANOVA)

Animals

Male Sprague–Dawley rats (~350g)

Animal Groups

- Control (n=5)
- Radiation 5 Gy (n=6)
- Radiation + Polytrauma (n=7)

Polytrauma Model

- Laparotomy
- Liver crush injury
- Hemorrhage (20% estimated blood volume)

Blood Collection

- Day 14 Post-Injury
- Femoral artery cannulation
- 2-3% Isoflurane anesthesia
- 100% oxygen supplementation

FSC (Forward Scatter) reflects cell size.
SSC (Side Scatter) reflects internal complexity or granularity.

Results

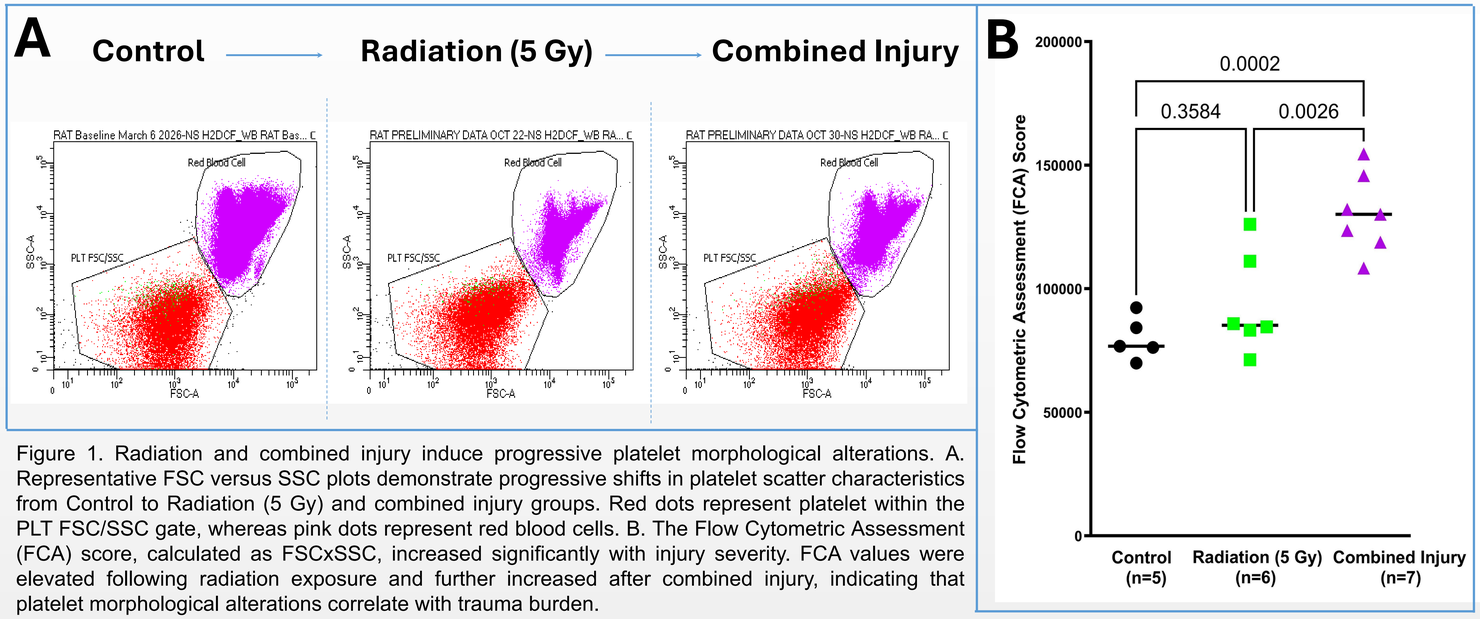

Figure 1. Radiation and combined injury induce progressive platelet morphological alterations. A. Representative FSC versus SSC plots demonstrate progressive shifts in platelet scatter characteristics from Control to Radiation (5 Gy) and combined injury groups. Red dots represent platelet within the PLT FSC/SSC gate, whereas pink dots represent red blood cells. B. The Flow Cytometric Assessment (FCA) score, calculated as FSCxSSC, increased significantly with injury severity. FCA values were elevated following radiation exposure and further increased after combined injury, indicating that platelet morphological alterations correlate with trauma burden.

- FCA increased progressively with injury severity. Compared with controls, FCA increased by **17.3%** following irradiation and by **63.3%** following combined injury. FCA was also **39.2%** higher in the CI group than in the IR group ($p<0.01$), demonstrating a strong association between platelet scatter characteristics and injury severity.
- Platelet counts decreased as FCA scores increased, indicating an inverse relationship.

Conclusion/Impact

- Progressive Increase: The FCA metric (derived from platelet FSC/SSC) increased progressively following radiation exposure and combined injury.
- Rapid, Label-Free Detection: Platelet scatter analysis successfully detects injury-associated morphological alterations without the need for staining.
- Translational Potential: The strong correlation between FCA and injury severity highlights its potential as a novel biomarker for battlefield triage and injury assessment.
- Next Steps: Future work will investigate temporal FCA dynamics and their direct relationship to post-injury platelet function.

Military Relevance

Rapid assessment of radiation combined injury remains a critical challenge in military and mass-casualty settings. FCA utilizes routine flow cytometric scatter measurements without additional reagents, offering a potential near-real-time biomarker for injury stratification and prolonged field care decision-making. This reagent-free approach may be particularly valuable in austere environments requiring rapid assessment.

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