Demographic Study Using the Cytokinesis-Block MicroNucleus (CBMN) Assay to Assess the Need for Separate Calibration Curves Among Different Demographic Groups in Determining Past Acute Radiation Exposure

Richard Kowalski[2], Ryan Mahnke[1], Johnny Ho[1], Matthew Snyder[1], Xiang Ma[1], Monta Whitney[1], Nariman Panahian[2], David Brenner[3], Guy Garty[3], Adayabalam Balajee[3], Helen Turner[3], Jay Perrier[3], Courtney Ashton[4], Evan Hempel[4], Douglas Henderson[1]; [1]Northrop Grumman Corp.; [2]Aerotek, Inc.; [3]Columbia University; [4]KAI-Research, Inc.

Abstract

The cytokinesis-block micronucleus (CBMN) assay has been proven as a reliable and quantitative assay in determining past acute radiation exposure levels. A demographic study using *ex-vivo* irradiated blood samples from human adults ranging from ages 21 to 79 was designed and executed to assess whether separate calibration dose response curves would be required for different demographic groups in the population. In addition to age and gender, a donor's smoking, Type II diabetes, alcohol and caffeine consumption status was also incorporated into the study design. A total of 242 donors were enrolled in the study. Peripheral blood samples (~5mL) were collected from healthy volunteers at a clinical research site after informed consent was obtained. Blood samples were shipped inside a temperature-controlled container to the Center for Radiological Research at Columbia University for CBMN analysis. Each blood sample was divided into 6 aliquots, with each aliquot receiving one of the following radiation doses (0, 1.5, 2.5, 5, 7.5, 10Gy) from a ^{137}Cesium source. Lymphocytes were isolated, cultured, cytokinesis-blocked, and fixed/stained. Imaging was carried out using an Olympus BX43 microscope with automated scoring of micronuclei (MN), mononucleates (MO) and binucleates (BN). Raw data from the CBMN assay was uploaded to KAI-Research, Inc. for archival and statistical analysis. Early statistical analysis confirmed definitive correlation between radiation dose and CBMN assay ratios of MN/BN and MO/BN. The preliminary analysis showed that there was no statistical difference between the different demographic groups with respect to assay results at each dose level. This indicates the CBMN assay is a robust, independent measure of radiation dose; eliminating the need for multiple calibration curves.

Participant Eligibility

Inclusion Criteria:
- Male or female adults aged 21-79 years.

Exclusion Criteria:
- Recipient of blood, organ or tissue product donation.
- HIV diagnosis or other disease or condition causing immune-compromise.
- Known blood borne active contagious disease.
- History of chemotherapy or radiation exposure in the past 3 years (aside from routine dental, DEXA scan and mammogram).
- Tobacco users who had recently quit.
- Non-smoking tobacco users.
- Non-smokers with routine exposure to second-hand smoke.
- Moderate alcohol or caffeine use.

Methods

Study procedures were conducted following ICH guidelines for Good Clinical Practice and all data were source-verified. Figure 1 outlines the CBMN assay:
- Whole blood (~5mL) was drawn into a lithium heparin anticoagulant blood collection tube.
- Blood samples were transported to Columbia University.
- Blood samples *ex vivo* gamma-irradiated.
- Lymphocytes were separated by density gradient centrifugation.
- Lymphocytes were incubated with 44 hours in growth medium containing phytohemagglutinin-M at 37ºC and 5% CO_2.
- Cytochalasin-B was added to each aliquot and samples were incubated for an additional 26 hours.
- Cells were washed with KCl and fixed with Methanol/Acetic Acid.
- Fixed cells were dropped on microscope slides air dried, and stained with DAPI.
- Stained cells were imaged using an Olympus automated microscope with customized digital scoring of mononucleated (MO), binucleated (BN) cells and numbers of micronuclei (MN).

Demographic Groups			Number of Participants		
Smoking	Diabetic Status	Age Group	Male	Female	Total
Non-smoker	Non-Diabetic	21-29	15	16	31
		30-39	16	15	31
		40-49	16	16	32
		50-59	16	16	32
		60-69	16	15	31
		70-79	15	9	24
	Type 2 Diabetic	50-59	16	13	29
Smoker	Non-Diabetic	40-49	17	11	28
Heavy Alcohol Users (≥5 drinks/week)		Various	19	13	32
Heavy Caffeine Users (≥340 mg caffeine/day)		Various	18	10	28

Table 1: Demographic Study Participant Enrollment Listing

Statistical Analysis

Data were analyzed using Multivariate Analysis (MANOVA) and a two-way ANOVA statistical model design followed by Bonferroni-Dunn and Scheffe posthocs. In addition to standard linear regression analysis to calculate the absorbed dose, machine learning algorithms (Table 2 and Figure 3) were employed to predict the dose based on multiple inputs (MN/BN, MO/BN, BN, MO, MN)

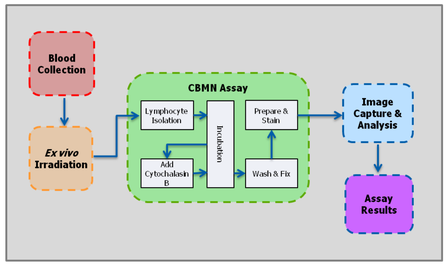


Figure 1: CBMN Assay Sample Processing & Analysis Flowchart

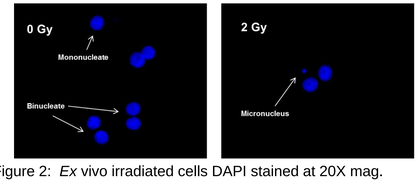
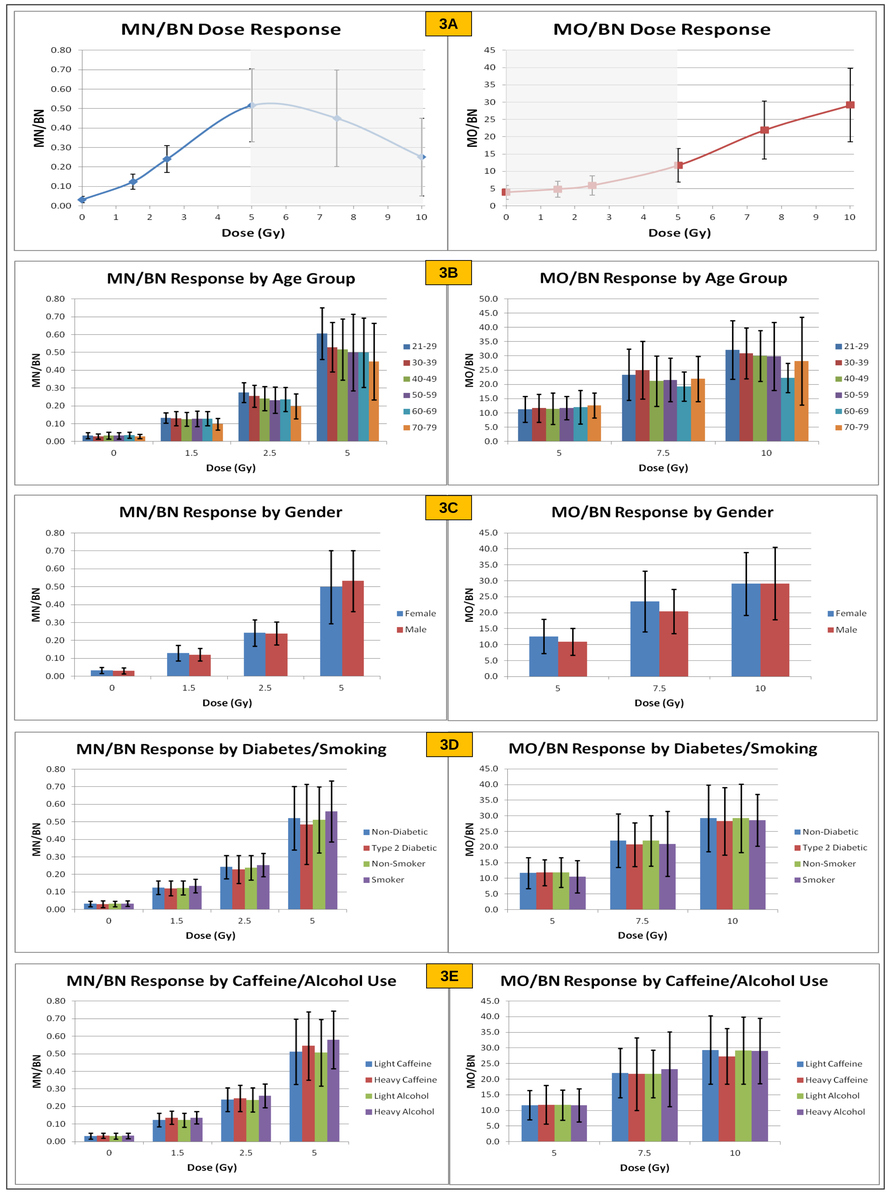


Figure 2: *Ex* vivo irradiated cells DAPI stained at 20X mag.

Results

Table 1 lists the demographics from 238 of the 242 participants enrolled. Four samples were excluded due to either extended shipping or culture contamination. Statistical analysis of MN/BN ratio for doses between 0 - 5 Gy and the MO/BN ratio for doses between 5 - 10 Gy (Figure 3A) confirmed a correlation between absorbed radiation dose and assay signal intensity ($p<0.001$). Graphs of both biomarkers for the various demographics tested are shown in Figures 3B to 3E. No statistically significant differences were observed across all of the demographics examined ($p>0.05$). Table 2 compares various methods to calculate the absorbed dose based on CBMN assay results. The legacy method refers to classical regression analysis as shown in Figure 3A while the other algorithms are various forms of machine learning tools. Of the algorithms tested, boosting regression showed the greatest concordance with known dose +/- 1 Gy. Figure 4 illustrates the data plotted as observed dose versus predicted dose for the Legacy method and Boosting regression method.



Figures 3A – 3E: MN/BN and MO/BN dose responses for various demographic groups studied

Table 2: Comparison of Dose Prediction Algorithms

Algorithm	Demographic Study Prediction Rate (+/- 1.0 Gy)
Legacy Method	*62%*
Neural Network	77%
MarSpline	71%
Regression Tree	74%
Boosting (regression)	78%
Tree Bag	75%

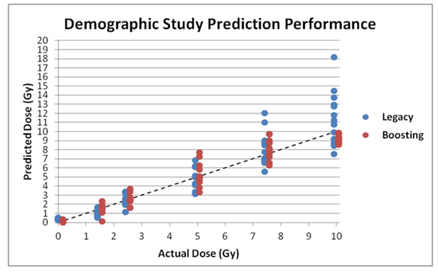


Figure 4: Linear Regression versus Boosting Regression

Summary

- The MN/BN and MO/BN ratios trended to lower values with older age groups; however, this trend did not impact calculations for predicting the dose received (Figures 3A and 4)
- The biomarkers (MN/BN and MO/BN) directly correlated with radiation dose ($p<0.001$) independent of the demographics examined
  - No statistical difference ($p>0.05$) was observed between the different demographic groups studied at each radiation dose level
- Use of machine learning algorithms improved dose prediction percentages from 62% to 78% within +/- 1 Gy
- Determination of an absorbed radiation dose using the CBMN assay is not likely to require multiple demographic dependent calibration curves. "One size fits all".

Acknowledgement

This project has been funded in whole or in part with Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, Department of Health and Human Services, under Contract No. HHSO100201000002C.