Original Research

System for Scoring Severity of Acute Radiation Syndrome Response in Rhesus Macaques (*Macaca mulatta*)

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We developed a clinical assessment tool for use in an NHP radiation model to 1) quantify severity responses for subsyndromes of the acute radiation syndrome (ARS; that is, hematopoietic and others) and 2) identify animals that required enhanced monitoring. Our assessment tool was based primarily on the MEDical TREatment Protocols for Radiation Accident Victims (METREPOL) scoring system but was adapted for NHP to include additional indices (for example, behaviors) for use in NHP studies involving limited medical intervention. Male ($n = 16$) and female ($n = 12$) rhesus macaques (*Macaca mulatta*; 5 groups: sham and 1.0, 3.5, 6.5, and 8.5 Gy; $n = 6$ per group) received sham- or bilateral ^{60}Co γ -irradiation at approximately 0.6 Gy/mn. Clinical signs of ARS and blood analysis were obtained before and serially for clinical assessment during the period of 6 h to 60 d after sham or ^{60}Co irradiation. Minimal supportive care (that is, supplemental nutrition, subcutaneous fluid, loperamide, acetaminophen, and topical antibiotic ointment) was prescribed based on clinical observations. Results from clinical signs and assays for assessment of relevant organ systems in individual animals were stratified into ARS severity scores of normal (0), mild (1), moderate (2), and severe (3 or 4). Individual NHP were scored for maximal subsyndrome ARS severity in multiple organ systems by using the proposed ARS scoring system to obtain an overall ARS response category. One NHP died unexpectedly. The multiple-parameter ARS severity scoring tool aided in the identification of animals in the high-dose (6.5 and 8.5 Gy) groups that required enhanced monitoring.

Abbreviations: ARS, acute radiation syndrome or sickness; METREPOL, MEDical TREatment Protocols for Radiation Accident Victims

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The currently accepted scheme to assess humans exposed to life-threatening radiation doses is the MEDical TREatment Protocols for Radiation Accident Victims (METREPOL). METREPOL is used to evaluate the time course and severity of radiation injury from acute radiation syndrome (ARS) on the basis of relevant organ-based systems to provide medical management guidance.²⁶ The METREPOL system is consistent with some of the pioneering work on radiation-induced clinical signs and symptoms.^{3,31–33} More recently, METREPOL has been updated for use in a 6-page Armed Forces Radiobiology Research Institute Biodosimetry Worksheet.^{65,66} The first verification of the effectiveness of the clinical dosimetry system METREPOL to rapidly and accurately predict later occurring ARS severity and to develop medical management strategies were based on real clinical data from case histories of accidentally exposed patients.²¹ Individual assessment of ARS severity response will allow medical responders to better manage irradiation-accident victims consistent with recommended guidelines.³⁴

Significant gaps exist to enhance radiologic medical countermeasures and biodosimetry capabilities in response to radiologic threats. One major gap is the ability to rapidly identify persons exposed to potentially life-threatening radiation doses and distinguish them from those not exposed or exposed to lower doses.¹² Biodosimetry devices for this purpose should be useful for triage applications involving mass-casualty exposure incidents and must obtain the necessary approvals by governmental regulatory agencies (that is, FDA).¹

Approval by appropriate regulatory agencies for the use of medical radiologic countermeasure drugs involves demonstration of safety and efficacy, ideally in humans. When human data are inaccessible, relevant animal models should be used. No biodosimetry devices are currently approved by the FDA, and approaches to obtain approval for candidate devices will likely be based on initial studies using small rodent models to supplement results from human patients undergoing radiation therapy for cancer and from limited radiation accidents.⁴² Because human data are quite sparse, a relevant nonrodent animal model—one that permits characterization of complete dose- and time-course responses—is needed for biodosimetry device validation.

NHP provide the bridge in scientific research from small laboratory rodents to humans for medical countermeasure and biodosimetry studies.^{4–7,18,19,24,39,48,69} NHP are physiologically

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and genetically similar to humans and can be managed to control for nongenetic potential confounders that can cause large interindividual variability in human populations. A cageside observation scoring system of total-body irradiation has been suggested for the assessment of the progression of clinical signs associated with ARS in mice.^{53,54} To our knowledge, an ARS scoring system has not been previously published nor applied for assessment of radiation injury severity in an NHP radiation model.

The purpose of the current study was to modify and apply an ARS severity scoring system to the NHP radiation model. This work is based on prior studies from University of Maryland collaborators.^{30,49} In addition, some assessment tools were taken from other colleagues, who have used NHP under other (for example, transplant) conditions.²³ Our goal was to establish a quantitative and harmonized approach to effectively assess the severity of multiple organ system-based ARS radiation injury, similar to that done in humans, as a refinement to our biodosimetry validation studies using NHP.

Materials and Methods

Ethics statement. The study (Protocol 2008-11-012 [Blakely]) was approved by the Armed Forces Radiobiology Research Institute's IACUC and underwent a required second-tier review by the Animal Care and Use Review Office of the US Army Medical Research and Materiel Command (USAM-RMC). Research with animals was conducted according to the principles enunciated in *Guide for the Care and Use of Laboratory Animals* (the *Guide*) prepared by the Institute of Laboratory Animal Resources, National Research Council, and was in compliance with the guidelines for animal welfare and amelioration of suffering as recommended in the Weatherall Report.^{41,73}

Animals. The FDA has accepted rhesus macaques as an appropriate animal model for testing radiation countermeasures under the Animal Efficacy Rule, where experiments cannot be performed in humans. All macaques in this study were housed in accordance with the *Guide* at the Armed Forces Radiobiology Research Institute (Bethesda, MD), which is AAALAC-accredited.⁴¹

This study included 16 male (weight, 4.3 to 9.0 kg) and 12 female (weight, 4.7 to 6.6 kg) Chinese-origin rhesus macaques (*Macaca mulatta*; age, 3 to 6 y; Primate Products, Miami, FL). Animals were transported and delivered by a climate-controlled truck in individual wooden crates with holes and a pan, along with a tray for food and water. On arrival, the macaques were quarantined for 45 d and underwent immediate and intermittent physical assessment prior to the study. Animals were serologically negative for simian retrovirus types 1, 2, 3, and 5; SIV, herpes B virus, and simian T lymphotropic virus 1 and tested negative for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and tuberculosis. The macaques were housed in individual stainless-steel, squeeze-back cages (floor area, 6 ft²; height, 32 in.) in conventional holding rooms at the Armed Forces Radiobiology Research Institute's Veterinary Sciences Department animal facility. Macaques had visual and tactile contact with an adjacent NHP and remained in the same rooms with their cohorts throughout the study. Environmental conditions in the animal room were maintained at 18 to 26 °C with 30% to 70% relative humidity, by using 10 to 15 air changes hourly of 100% conditioned fresh air and a 12:12-h light:dark cycle (lights on, 0600; 320 lux at 1 m above the floor). All animals were fed 130 g of autoclaved, commercially available primate diet (Teklad Certified Global 20% Protein Primate Diet 2050, Harlan Teklad

Animal Diet and Bedding, Madison, WI) twice daily and received enrichment in the form of disinfected fresh fruits and vegetables, dried fruits, or foraging mix once daily. Tap water was freely available to all macaques. Each animal was provided with various manipulanda, such as commercially available toys and mirrors, and all had access to observe DVD broadcasts.

Experimental design. The macaques were match-paired as closely as possible by weight and sex into 5 groups of 6 animals group (sham irradiation and 1.0, 3.5, 6.5, and 8.5 Gy) and received bilateral ⁶⁰Co γ-irradiation at approximately 0.6 Gy/min (or sham irradiation). Use of the ⁶⁰Co γ-ray source at the Armed Forces Radiobiology Research Institute was justified due to its ability to achieve uniform exposures of large animals through bilateral irradiation. This source has a half-life of 5.27 y, thus affording a practical and sustained source dose rate for examining photon radioresponses at our institute. The dose rate of approximately 0.6 Gy permits accurate exposures over a range of doses (0, 1, 3.5, 6.5, and 8.5 Gy) spanning 1.6 to 14.2 min. Two macaques that underwent sham irradiation later served as experimental animals in the 8.5-Gy group. Radiotelemetry chips (Bio Medic Data Systems, Seaford, DE) were implanted subdermally by injection in all of the animals, to permit radiotelemetry-based measurement of subdermal temperatures. Blood was withdrawn and ARS severity response scores were obtained before (that is, 2 to 6 d prior to irradiation) and serially as needed for clinical assessment during the period of 6 h to 60 d after irradiation. Blood typically was not collected nor was ARS severity response scored on weekends or holidays, unless an animal was identified as requiring enhanced monitoring for potential humane euthanasia. However, the NHP were observed 2 or 3 times daily during these periods and the veterinarian on-call was kept apprised of the health status of the NHP. Values for the ARS severity score sheet were entered as soon as possible after the data were obtained or observed. The score sheets were up-to-date for the veterinarian on-call for the weekend or holiday.

Radiation exposure and dosimetry. In vivo radiation exposures and dosimetry of rhesus macaques were performed similarly to that previously described.^{10,59} All irradiated NHP were fasted overnight during the evening prior to irradiation. The nominal dose rate was 0.6 Gy/min, but the measured dose rates ranged from 0.54 to 0.56 Gy/min (mean ± SEM, 0.55 ± 0.01 Gy/min). During irradiation of each group, 2 macaques were sham-irradiated (0 Gy), except for the groups receiving 1.0 or 6.5 Gy; these 2 cohorts were irradiated on the same day, and the same pair of macaques served as the sham controls for both. The doses were selected as part of a dose-response study designed to evaluate various candidate biodosimetry assays. Animals were anesthetized (10 mg/kg IM ketamine) for the entire radiation procedure, placed in a Plexiglas restraint chair, and were irradiated. Dosimetry was performed by using an alanine-electron paramagnetic resonance system, with calibration factors traceable to the National Institute of Standards and Technology (Gaithersburg, MD) and confirmed through an additional check against the standard ⁶⁰Co source at the UK National Physics Laboratory (Teddington, Middlesex, United Kingdom).

Monitoring before and after irradiation. We developed an ARS severity scoring system, which was modeled after that developed previously for humans²⁶ but was modified for the rhesus macaque radiation model, and used it in the current study. Our ARS severity scoring systems involves measurement of clinical signs and symptoms for relevant ARS subsyndromes (that is, gastrointestinal, neurovascular, respiratory, cutaneous, and hematopoietic systems). The degree of severity for each quantitative (that is, measurable values, including CBC, body

weight, serum osmolality) and qualitative (that is, observable, including vomiting, diarrhea, lethargy) parameter was annotated as 0 (none), 1 (mild), 2 (moderate), or 3 or 4 (severe).

To acclimate the NHP to the observer and to standardize the process, a single observer evaluated the animals daily in the following manner. The observer, who was fully gowned, double-gloved, and masked, entered the room and avoided direct eye contact with the NHP. The observer first noted the general activity level of the animals: whether they were active or passive on the observer's entry, their general state of alertness (doing flips or swinging from the top bars of the cages), and their degree of vocalization. The observer looked for any signs of vomitus, diarrhea, and blood in the pans below the cage. In addition, the observer searched for any signs of external injury to the limbs. The observer gave the NHP treats and snacks (especially kiwi fruit) to determine their level of excitement and hunger. Respirations were counted on the individual animals. The data were entered into the ARS severity scoring sheets (not shown); a digital version of the ARS severity scoring sheets can be accessed by contacting the corresponding author. Animals were observed twice daily during the first 2 wk after irradiation and at least once daily during the work week thereafter. The data on these scoring sheets, along with hematology and other results, were transcribed into electronic spreadsheets.

The subsyndrome ARS scores for individual animals were based on the highest score for parameters within each of the subsyndromes (gastrointestinal, neurovascular, and hematopoietic; Figure 1), similar to what is done in METREPOL. The ARS response category for an individual animal represents the maximal ARS severity observed (Figure 1).

At scheduled serial biosampling time points, body weights were obtained from NHP collar-trained to chair themselves or during ketamine anesthesia. Blood samples were obtained immediately after animals were weighed. Subdermal body temperatures were measured by using a previously implanted radiotelemetry chip. All values on the ARS severity score sheet were recorded as soon as possible after obtained. Using our ARS severity scoring template (data not shown) the subsyndrome as well as the overall maximal severity score was recorded in addition to peak scores for each animal. The overall RC for an individual animal represents the maximal severity score among all of the subsyndrome organ systems.

Hematology and blood chemistry. Peripheral blood samplings in the rhesus macaque *ex vivo* radiation model system were performed similar to that previously described.¹⁰ Blood biosampling was limited to no more than 1% of the animal's total blood volume at any given time. Peripheral blood (less than 1.5 mL) was drawn before and after irradiation from a saphenous vein (alternated daily) in conscious chaired or ketamine-anesthetized animals by using heparin-wetted needles (23 gauge), potassium-EDTA vacuum phlebotomy tubes (catalog no. 365974, Becton Dickinson, Franklin Lakes, NJ), and serum separator tubes (catalog no. 365967, Becton Dickinson). Blood in EDTA tubes for CBC measurements were analyzed within several hours after sampling. Hematology values were obtained by using an Advia 2120 (Siemens Healthcare Diagnostics, Deerfield, IL), and blood chemistry values were determined by using a Vitros 250 (Ortho-Clinical Diagnostics, Rochester, NY). Serum osmolality (mOsm/kg H₂O; Ektachem Analyzer, Kodak, Rochester, NY) was used to determine hydration status and was estimated according to the following equation:^{8,37}

$$[1.86 (\text{Na}^+ \text{ in mg/dL}) + (\text{glucose in mg/dL}) + (\text{BUN in mg/dL})] / 2.8.$$

Treatment after irradiation and euthanasia. This study design was modeled to permit assessment of radiation responses prior to use of supportive-care medical treatment. A second goal of the study was to characterize the time course for biomarkers of acute phase and ARS organ injuries expressed during the evolution of the clinical case (0 to 60 d). Full supportive-care treatments involve intravenous injections that are likely to affect the plasma levels of these biomarkers and hematologic parameters. Thus the macaques received only minimal supportive care, such as supplemental nutrition (that is, oral electrolyte solutions), subcutaneous fluids (that is, saline), loperamide (1 mg PO BID), acetaminophen (5 to 10 mg/kg PO TID), and antibiotic cream to ameliorate discomfort due to skin lesions; treatments were given only as needed according to clinical observations and ARS severity scores. Previous studies have followed a similar minimal approach regarding supportive care in NHP.^{4,69} All surviving animals in the current study were returned to the Armed Forces Radiobiology Research Institute Veterinary Science Department's holding facility.

The criteria for euthanasia were any one of the following: 1) weight loss greater than 20% over a 3-d period; 2) inappetence, defined as anorexia for 3 d concurrent with deteriorating clinical signs; 3) weakness or inability to obtain feed or water concurrent with an inability or reluctance to stand over more than 24 h; 4) minimal or absence of response to external stimuli; 5) core body temperature less than 35.9 °C for 2 consecutive readings more than 6 h apart with a period of febrile neutropenia (absolute neutrophil count of 0.5 cells/μL or less); 6) severe acute anemia (Hgb, less than 40 g/L; Hct, less than 13%); 7) severe dyspnea or cyanosis; 8) severe (life-threatening) vomiting or diarrhea, obstruction, intussusception, peritonitis, or evisceration; 9) CNS depression, seizures, or paralysis; 10) nonhealing wounds, repeated self-trauma, or severe skin infections; and 11) severe thrombocytopenia (less than $20 \times 10^3/\mu\text{L}$). Macaques were euthanized with an intravenous overdose of sodium pentobarbital (1 mL/4.5 kg; Euthasol, Virbac Animal Health, Fort Worth, TX), as recommended in the AVMA guidelines.²

Statistics. When designing this experiment, we performed a preliminary analysis for planned outcome variables, which indicated that a sample size of 6 would provide 80% power to detect a difference between any 2 groups. At study end, we evaluated selected qualitative (that is, comparison of controls with 6.5-Gy-irradiated NHP; inappetence ARS severity, diarrhea severity) and quantitative (lymphocyte and neutrophil counts at day 9) measures and confirmed that this assumption regarding statistical power was correct (data not shown). All group values are given as mean $\pm$ SE (or 1 SD), as appropriate. The shaded areas in Figures 2 and 3 represent the 95% CI from the values of the pooled preirradiation (baseline) blood withdrawals from individual animals. Fit lines and equations were determined by using SigmaPlot 12 (Systat Software, San Jose, CA). *P* values of 0.05 or less was considered to be significant.

Results

In the current study we monitored the severity of ARS in rhesus macaques during the early (or prodromal), latent, manifest illness, and recovery or death (humane euthanasia) phases by evaluating various relevant organ systems (that is, hematopoietic, gastrointestinal, neurologic or vascular, respiratory, and cutaneous) in a total-body irradiation dose-response model according to the convention described by METREPOL.²⁶

Hematopoietic system. Radiation caused dose- and time-dependent changes in the hematopoietic system. Taken from each animal at 3 d before irradiation, control values (mean [95% CI]) for

	Normal (score, 0)	Mild (1)	Moderate (2)	Severe (3 or 4)
Gastrointestinal system				
Dehydration				
Weight loss (%)	± 5	10	15	20
Skin turgor (anesthesia)	1 s	2 to < 3 s	3 s	>3 s
Gum capillary refill (anesthesia)	1 s	2 to < 3 s	3 s	>3 s
Buccal mucosal and tongue hydration (anesthesia)	Normal	Less than normal amount of saliva, yet still some shine to membrane, tacky	Dry, no saliva, mucus membranes not shiny, tacky, sunken eyes	Dry and inflamed, tongue parched
Retching or vomiting	None	Mild, some evidence of vomitus (that is, <5 mL) on cage floor	Moderate, much evidence of vomitus (that is, ≥5 mL) on cage floor	Severe, actively retching or vomiting on inspection
Inappetence	Normal eating habits, consumes all or most of food	Moderately slow to respond to meals and treats	Portions (>50%) of meal and treat remain uneaten, picks up food but may abandon it	Ignores food when delivered, all (or most) of food remains uneaten
Diarrhea				
Abdominal distension	None	Mild, ≤500 mL volume increase	Moderate, 500 to 1000 mL volume increase	Severe, >1000 mL
Stool consistency	Formed	Soft	Loose watery	Bloody watery
Stool color	Normal	Other (for example, green, yellow)	Black	Bright red
Neurovascular system				
Response to stimuli	Vigilant and reactive; stands on perch inside cage; looks around room; follows personnel and procedures	Vigilant and mildly reactive: stands on perch, looks around room; follows personnel and procedures	Generally sits on perch; appears depressed; moves with difficulty when provoked	Always sits on the perch or floor of cage, even with personnel present; appears depressed (unresponsive; lies on floor or perch)
Fever				
Rectal body temperature with anesthesia	Approximately 38.6 °C	Approximately 39.7 °C	Approximately 40.6 °C	>41.7 °C
Shivering	None	Mild, <20% body affected	Moderate, >20% to <50% of body affected	Severe, >50% of body affected
Piloerection (unrelated to fear or aggression)	None	Any	Not applicable	Not applicable
Behavioral				
Fur grooming	Good (groomed)	2 areas not groomed	3-4 areas not groomed	Not groomed
Aggression and vocalization	No sounds, calm	Makes sound once or twice	Sounds twice or more	Frequent sounds
Respiratory system				
Respiratory rate (breaths per min)	35-50	>60	>60 and shallow	Labored or gasping
Hematopoietic system				
Lymphocytes (reference value, ×10 ⁹ cells/L)	95% normal range: 1.04-6.87	1-14 d: 1.0-01.85	1-14 d: 0.45-1.0	1-14 d: <0.4
Neutrophils (reference value, ×10 ⁹ cells/L)	95% normal range: 0.04-5.23	<1d: ≥13 10-20 d: 1.2-1.6	<1 d: 11-13 10-24 d: <1.2	<1 d: 8-11 5-20 d: <1.0
Platelets (reference values, ×10 ⁹ cells/L)	95% normal range: 195-449	≥12 d: 180-250	≥8 d: <180	≤12 d: <180
Hemoglobin (reference value, g/dL)	95% normal range: 10.07-13.51	>20 d: 8-10.9	10-20 d: 8-10.5 ≥20 d: <8	≤10 d: <10 10-20 d: <8
Hct (reference value, %)	95% normal range: 29.4-39.9	>20 d: 27.0-32.4	10-20 d: 25-30 ≥20 d: 20-27	≤10 d: <30 10-20 d: ≤25

Figure 1. Grading system for rhesus macaque ARS response of gastrointestinal, neurovascular, respiratory, and hematopoietic systems.

lymphocytes, neutrophils, platelets, Hgb, and Hct were $3.95 (1.04 \text{ to } 6.87) \times 10^9/\text{L}$, $2.64 (0.04 \text{ to } 5.23) \times 10^9/\text{L}$, $322 (196 \text{ to } 449) \times 10^9/\text{L}$, $11.8 (10.1 \text{ to } 13.5) \text{ g/dL}$, and $34.7\% (29.4\% \text{ to } 39.9\%)$, respectively.

The time course changes in lymphocytes (Figure 2), neutrophils (Figure 2), platelets (Figure 2), Hgb (Figure 3), and Hct (Figure 3) are shown for each irradiated dose cohort of NHP. The shaded area in each panel of Figures 2 and 3 represents the 95% CI of pooled preirradiated values of each hematologic parameter.

In the macaques exposed to 1.0 Gy, lymphocyte counts (Figure 2 D), Hgb (Figure 3 C), and Hct (Figure 3 D) remained within the 95% CI throughout the entire 60-d monitoring period. Neutrophil counts in this group (Figure 2 E) showed the classic rise immediately after irradiation and returned to values within the 95% CI for the remainder of the 60 d. In contrast, platelet counts after 1.0 Gy (Figure 2 F) began to fall at day 10 postirradiation, reaching a low-normal nadir at day 18 after irradiation and returning to within the 95% confidence levels by day 22.

In animals that received 3.5 Gy, mean lymphocyte counts (Figure 2 G) fell below the 95% CI on day 1 after irradiation, remained below the lower 95% CI for about 20 d, and then returned to low-normal for the remainder of the study. Except for the rise at 4 h, mean neutrophil values (Figure 2 H) fell to the low-normal limit of the 95% CI after day 10, remained near or below this level until day 24, and then returned to the normal range. The platelet count (Figure 2 I) fell below the 95% CI by day 9, reached a nadir at day 17, and recovered to within the lower CL by day 23. Hgb (Figure 3 E) fell below the lower limit

of the 95% CI at day 10, showed a second phase drop at day 21, and then slowly recovered to within the lower CI limit at 49 d after irradiation. Like Hgb, Hct (Figure 3 F) showed a 2-phase drop below the 95% CI at days 9 and 21, with a slow return to within the 95% CI by day 49.

All hematology values for those animals that received 6.5 or 8.5 Gy fell below the 95% CI after irradiation and never returned to baseline. This drop occurred on day 1 for lymphocytes (Figure 2 J), day 7 for neutrophils (Figure 2 K), between days 5 and 8 for platelets (Figure 2 L), and day 9 for Hgb (Figure 3 G) and Hct (Figure 3 H).

During the experimental planning phase, we defined distinguishing blood parameter ranges at specific time points to characterize different hematopoietic ARS severity levels (Figure 1). Minor changes to these criteria were made at the data analysis phase. Once blood parameters for an irradiated NHP fell into a hematology criterion (box, Figures 2 and 3), the NHP was scored at that specific hematopoietic severity level. The hematopoietic maximal severity levels for individual animals are shown in Table 1. Sham animals showed hematopoietic maximal severity levels of 0, 0 or 1, or 1, illustrating that normal levels can overlap with hematopoietic severity level of 1 (Table 1). Individual NHP exposed to 1 Gy exhibited hematopoietic maximal severity levels of 2 and 3 or 4 (Table 1). In this case, the overall time course pattern of 1-Gy-exposed NHP shown by the mean values of 6 replicates was consistent with a level 1 severity (Figures 2 and 3), but excursions at a few time points for individual animals caused the increase in a scoring of the maximal responses shown in Table 1.

Figure 2. Time course of changes in blood cell counts (mean $\pm$ SEM; $n = 6$ per cohort) in a NHP radiation dose-response model. (A, D, G, and J) Lymphocytes, (B, E, H, and K) neutrophils, and (C, F, I, and L) platelets from (A through C) sham-irradiated animals or irradiated to (D through F) 1.0 Gy, (G through I) 3.5 Gy, or (J through L) 6.5 or 8.5 Gy. The shaded areas represent the background (preirradiated; $n = 30$) values $\pm$ 95% CI; c, preirradiation control ($n = 6$). The boxes drawn in panels D through L represent the ranges used in the ARS sheet (Figure 1) to determine degree of severity.

The overall hematopoietic time-course changes for 3.5-Gy-irradiated NHP were generally consistent with level 2 severity (Figures 2 and 3). However individual NHP showed

hematopoietic excursions that caused the maximum ARS severity levels to rise to scores of 2 and 3 or 4 (Table 1). The time-course pattern of hematopoietic parameters for NHP irradiated

Figure 3. Time course of changes in Hgb and Hct (mean $\pm$ SEM; $n = 6$ per cohort) in an NHP radiation dose-response model. (A, C, E, and G) Hgb and (B, D, F, and H) Hct from (A and B) sham-irradiated animals or exposed to (C and D) 1.0 Gy, (E and F) 3.5 Gy, or (G and H) 6.5 or 8.5 Gy. Shaded areas represent the background (preirradiated; $n = 30$) values $\pm$ 95% CI; c, preirradiation control ($n = 6$). Boxes drawn in panels C through H represent the ranges used in the ARS sheet (Figure 1) to determine degree of severity.

with 6.5 or 8.5 Gy were consistent with severity scores of 3 or 4 (Figure 1). All individual animals in both the 6.5 and 8.5 Gy dose groups reached maximal severity scores of 3 or 4 (Table 1).

Gastrointestinal system. Indicators of gastrointestinal distress comprised emesis (Figure 4 A), diarrhea (Figure 4 B) and inappetence (Figure 4 C). These graphs show a strong concordance

Table 1. ARS subsyndrome severity levels; response category maximum, onset, and duration; and time of death or euthanasia in individual macaques

Dose (Gy)	Animal ID	ARS subsyndrome (maximal severity score)					Response category			Time of death or euthanasia (no. of days after irradiation)
		G	N	R	C	H	ARS severity (highest response category)	ARS onset (no. of days after irradiation)	ARS duration (d)	
Sham										
	E24	1	2	0	0	0 or 1	1	9	1	not applicable
	E23	0	0	0	0	1	1	14	25	not applicable
	E22	0	0	0	0	0	0	not applicable	not applicable	not applicable
	E21	0	0	0	0	0 or 1	0 or 1	1	24	not applicable
	E08	0	0	0	0	0	0	not applicable	not applicable	not applicable
	E07	0	0	0	0	0	0	not applicable	not applicable	not applicable
1.0										
	E14	0	0	0	0	3 or 4	3 or 4	0	31	not applicable
	E13	0	0	0	0	3 or 4	3 or 4	0	60	not applicable
	E12	1	0	0	0	2	2	0	60	not applicable
	E11	0	3 or 4	0	0	2	2	1	60	not applicable
	E10	0	1	0	0	2	2	1	59	not applicable
	E09	1	1	0	0	3 or 4	3 or 4	0	60	not applicable
3.5										
	E06	0	0	0	0	3 or 4	3 or 4	0	60	not applicable
	E05	1	0	0	0	3 or 4	3 or 4	0	60	not applicable
	E04	1	0	0	0	3 or 4	3 or 4	0	60	not applicable
	E03	1	0	0	0	3 or 4	3 or 4	0	60	not applicable
	E02	0	0	0	0	2	2	0	60	not applicable
	E01	0	0	0	0	3 or 4	3 or 4	0	60	not applicable
6.5										
	E20	3 or 4	3 or 4	0	0	3 or 4	3 or 4	0	18	18
	E19	3	0	0	0	3 or 4	3 or 4	0	16	16
	E18	2	2	0	0	3 or 4	3 or 4	1	13	14
	E17	3 or 4	2	0	0	3 or 4	3 or 4	0	14	14
	E16	3 or 4	3 or 4	0	0	3 or 4	3 or 4	0	16	16
	E15	2	1	0	0	3 or 4	3 or 4	0	14	14
8.5										
	E30	3 or 4	2	0	0	3 or 4	3 or 4	0	11	11
	E29	3 or 4	2	0	0	3 or 4	3 or 4	0	11	11
	E28	3 or 4	3 or 4	0	0	3 or 4	3 or 4	0	11	11
	E27	3 or 4	2	0	0	3 or 4	3 or 4	0	12	12
	E26	3 or 4	3 or 4	0	0	3 or 4	3 or 4	2	12	14
	E25	3 or 4	2	0	0	3 or 4	3 or 4	3	11	14

C, cutaneous; G, gastrointestinal; H, hematopoietic; N, neurovascular; R, respiratory

or correlation between the percentage incidence and the ARS severity scores for each of these measures throughout the irradiation dose range. The percentages of emesis, diarrhea, and inappetence were radiation-dose-dependent according to r^2 values of 0.991, 0.926, and 0.957, respectively, determined by using the equation shown in Table 2. Likewise the ARS scores for emesis, diarrhea, and inappetence were radiation-dose-dependent (r^2 : 0.998, 0.988, 0.995, respectively).

All of the emetic episodes occurred within a few hours after irradiation, and the responses were radiation-dose-dependent according to both percentage of occurrence and ARS severity scores (Figure 4 A). However, one NHP in the 8.5-Gy-irradiated group also vomited on the second day after irradiation. Diarrhea (Figure 4 B) in the animals irradiated with 6.5 Gy (4 of 6 animals) and 8.5 Gy (6 of 6) began 4 d after irradiation, and all of these NHP were treated with loperamide (0.5 mg BID in a

treat) twice daily for 5 d until the diarrhea resolved. In addition, the animals received cubes of frozen sports drink (Gatorade, Pepsico, Harrison, NY) twice daily during this time interval. A few NHP irradiated at lower doses had diarrhea scores of 1 (loose stools), and none of the sham-irradiated animals had diarrhea.

An observer noted that animals with diarrhea after irradiation with 6.5 Gy began to show diminished appetites (Figure 4 C) that appeared to precede their need for euthanasia. We therefore developed a scoring system for this symptom and added it to the ARS worksheet (Figure 1). Only 3 of the 6 animals in this group were assigned an ARS score in this category, but the other 3 showed the symptom as well (data not shown). All of the animals in the remaining groups were scored. Only one animal in the sham-irradiated group showed mild (score, 1) inappetence, and this event occurred toward the end of the first

Figure 4. Gastrointestinal ARS parameters in an NHP radiation dose-response model. Dose response for incidence (left y-axis; filled circles) and ARS severity score (right y-axis; open circles) for (A) emesis, (B) diarrhea, and (C) inappetence after sham irradiation or exposure to 1.0, 3.5, 6.5, or 8.5 Gy. Data are given as mean $\pm$ SEM ($n = 4$ to 6 animals per dose cohort)

30 d. Likewise, 1 and 2 animals, respectively, in the 1.0- and 3.5-Gy-irradiated groups showed mild to moderate inappetence. Again, this symptom occurred only once and toward the end of the first 30 d of observation. In contrast, for those animals receiving higher irradiation doses (that is, 6.5 and 8.5 Gy), inappetence was severe (score, 3) and began 1 to 4 d before euthanasia was elected.

Figures 5 and 6 illustrate values from 2 other parameters gathered to assess the effects of radiation on the gastrointestinal system: body weight (Figure 5) and serum osmolality (a measure of hydration; Figure 6). When weight was normalized to control (preirradiation) weights before being plotted, on average none of the animals lost a significant amount of body

weight during the study (Figure 5). Conversely, all groups of animals, in general, showed an increase in body weight over the duration of the experiment. Occasional changes and reductions in body weight of no more than 5% occurred, but these involved single time points, and typically body weight returned to the preirradiation value by the following weight measurement. Each reduction likely was associated with a particular procedure or possibly the time of day that the animal was weighed. In addition, all of the animals appeared to be well hydrated because normal serum osmolality was maintained in all irradiated NHP throughout the study (Figure 6). Normal values were obtained from sham-irradiated and at preirradiation. The normal range for serum osmolality in this study was 275 to 333 mOsm/kg (mean $\pm$ SE, 290.75 $\pm$ 0.732 mOsm/kg).

For each dose cohort, individual NHP were scored for maximal gastrointestinal ARS severity (Table 1). Over this range of doses, a gradient of maximal severity emerged. At 1 Gy, 2 of the 6 NHP showed a maximal severity score of 1. After 3.5 Gy, 3 of the 6 NHP exhibited a maximal severity score of 2. In the case of the NHP that received 6.5 or 8.5 Gy, 9 of the 12 animals exhibited a maximal severity of 3 or 4. In addition, 3 of the NHP exposed to 6.5 Gy and 5 of those irradiated at 8.5 Gy showed a clear biphasic time-course pattern for gastrointestinal ARS severity (data not shown).

Neurovascular system. Response to stimuli. The grading system for this sign was adapted from a previous study.²³ With regard to the sham-irradiated animals and those receiving the lower 2 irradiation doses, there were only a few (1 or 2) isolated and brief instances of mild to severe lack of responsiveness to stimuli in 0 to 3 animals per group (Table 1). In 3 animals irradiated with 1 Gy, this event was on the day after irradiation. The other episodes for this group were on day 15 of the study and were only mild. This pattern stands in stark contrast to NHP that received 6.5 or 8.5 Gy, for which all but one animal showed mild to severe lack of responsiveness immediately after irradiation and on the following day. The response levels temporarily returned to normal until the animals' health began to deteriorate considerably, at which point, euthanasia was usually warranted within a few days.

Body temperature. The body temperatures of the NHP typically were taken daily until day 30 after irradiation; thereafter temperatures were measured every 2 to 3 d for the remaining 30 d. The body temperatures were recorded from either the implanted microchips or by using a rectal thermometer and sometimes by both methods. When both recording methods were used, only the rectal temperatures were used for calculations. The body temperatures of the control group ranged from 37.2 to 40 °C over the 60 d of the study. For animals in the 1.0- and 3.5-Gy groups, temperature ranges over 60 d were 36.2 to 40.4 °C and 36.9 to 40.4 °C, respectively. For NHP in the 6.5- and 8.5-Gy groups, temperatures ranged from 36.2 to 40.4 °C and 36.9 to 40.4 °C, respectively. Given that animals in the 6.5- and 8.5-Gy groups respectively survived for 18 and 11 d, control body temperatures taken over these time intervals ranged between 37.7 to 39.8 °C and 38.1 to 39.8 °C. Therefore body temperatures did not differ between any animals or among any of the groups. There were no significant or radiation-dose-dependent temperature changes after irradiation.

Respiratory system. Throughout the study, the respiratory rates of all animals in all groups ranged from 30 to 40 breaths per minute.

Cutaneous system. No wounds, injuries, or local infections occurred in the sham-irradiated group or those that received

Table 2. Coefficients, intercepts, and correlation coefficients for fitted curves of data shown in Figure 3

		Correlation coefficient	Coefficients of fitted equation				Intercept of fitted equation	
		r ²	a	SE	b	SE	X ₀	SE
Occurrence (%)	Emesis	0.991	104.1	6.47	1.777	0.299	3.502	0.346
	Diarrhea	0.926	273.9	875.3	3.626	3.267	10.53	19.62
	Inappetence	0.957	113.7	24.29	1.614	0.768	4.325	1.238
ARS (mean score)	Emesis	0.998	3.067	0.137	1.225	0.119	5.435	0.198
	Diarrhea	0.988	3.555	0.643	1.35	0.408	6.196	0.691
	Inappetence	0.995	3.063	0.185	0.876	0.178	4.871	0.876

Equation for curve fits: $y = \alpha / [1 + e - ((X - X_0) / b)]$

1.0 or 6.5 Gy. In the 3.5-Gy group, one animal had a mild (score, 1) laceration on a digit, and a topical antibiotic was applied on days 18 through 25. Within the 8.5-Gy irradiated group, one animal had a mild (score, 1) on day 9 after irradiation, and another had mild to moderate hemorrhage (score, 1 or 2) from its gums on days 8 through 11 d after irradiation, which by day 11 had returned to a mild score (1).

Scoring system for ARS severity in NHP. The radiation effect in this dose–response study (0 to 8.5 Gy) spanned a broad range of radiation injury levels, which were the basis for creating an ARS severity scoring scale (Figure 1) and scoring sheet (data not shown) for the relevant organ systems. Acute effects of these ARS were exhibited in and stratified according to these organ systems. The data values for the various endpoints (Figure 1) were selected to distinguish between the different levels of severity and were often based on differences in onset time and duration as well as the magnitude of the change in irradiation response. The normal values (95% CI) for hematology parameters were derived from mean baseline values, and those for body weights, osmolality, and temperatures were derived from baseline and sham measurements. The definition of normal (severity score, 0) for NHP appearance was established by observing all animals prior to the study and by monitoring the cohort of sham-irradiated animals throughout the study.

Individual NHP were scored for maximal ARS subsyndrome severity, which showed predominance of the hematopoietic system in contributing to the overall ARS response category (Table 1). NHP exposed to the higher doses (6.5 and 8.5 Gy) also exhibited significant gastrointestinal system severity. The onset and duration of ARS as well as the time of death (6.5 Gy, 14 to 18 d after irradiation; 8.5 Gy, 11 to 14 d after irradiation) are consistent with the contribution of hematopoietic ARS to death (Table 1).

Discussion

ARS, which involves multiple organ systems, can be confounded due to biphasic responses in specific organ systems. For example, our current NHP study shows that the responses of inappetence (gastrointestinal subsyndrome) and reaction to stimuli (neurovascular subsyndrome) exhibited biphasic responses (data not shown). The METREPOL scaling system accounts for multiple organ systems, and at a recent European consensus meeting, this severity scoring system scale was recommended for use on hospitalized radiation-accident victims after screening for nonirradiated bystanders and outpatient candidates.³⁴ However, harmonization or consensus similar to the METREPOL ARS severity scoring system for use in animal radiation models is unavailable currently. The data

presented here validate that this harmonization can be accomplished and using such an ARS severity assessment system in animal models supports the principles of refinement in humane animal studies.

Herein we have introduced an ARS severity scoring system and applied it in the rhesus macaque radiation model (Figure 1 and Table 1). The results showed that an ARS severity scoring sheet was successfully adapted for use in NHP to accurately assess the health status of the animals after recent irradiation dosage. The scoring sheet, adapted from that suggested for medical management of suspected radiation-exposed humans,²⁶ incorporated a combination of quantitative values (hematology, osmolality, body weight) and qualitative (vomiting, diarrhea, inappetence). Such combined assessment criteria are precisely what were used for a study of pig-to-NHP renal xenotransplantation.²³

Our ranges of control values for neutrophils and platelets are similar to but somewhat narrower than those reported previously.⁶⁹ In that study, doses of 6 Gy ⁶⁰Co or 6 MV X-ray were considered to be the LD_{50/30} (that is, the radiation dose associated with 50% survival at 30 d after irradiation) for survival. The authors reported that the accumulation of days of severe thrombocytopenia (less than 20 cells/μL) until day 14 (on which the first death occurred) was more predictive of mortality than were days associated with neutropenia (fewer than 0.5 cells/μL). Likewise, in our current study, early (before 14 d postirradiation) severe thrombocytopenia was a strong indicator for mortality and correlated with animals that showed internal hemorrhage (data not shown).

In another study, NHP irradiated with 6.7-Gy ⁶⁰Co (considered the LD_{50/30} survival value) showed thrombocytopenia beginning at day 6 after irradiation, with the nadir at day 14 and recovery by day 20.⁴ Leukocyte suppression began at day 2 and lasted until day 22, with the nadir at day 14. In our study, animals that received 3.5 Gy of irradiation had thrombocytopenia that began on day 10, reached the nadir on day 18 or 19, and recovered by day 25. In an NHP irradiation study in which medical support was provided, the absolute neutrophil count fell below 500 cells/μL beginning 3 to 5 d after irradiation and dropped to less than 100 cells/μL within 5 to 6 d, whereas platelets were less than 20,000 or 10,000 cells/μL on average between 8.6 and 9.7 d postirradiation; shortly afterward, transfusions began.²⁴ The irradiation doses for that study ranged from 7.2 to 8.9 Gy and, with full supportive-care treatment, neutrophil counts recovered in surviving animals.²⁴ Progressively higher doses of radiation cause incrementally increased damage to the bone marrow and injure the stem cells that produce platelets, resulting in the pattern of decreasing platelet count, nadir, and subsequent return to normal values in peripheral blood. In our

Figure 5. Time course of percentage change in body weight in an NHP radiation dose-response model. Time course of percentage change in body weight after (A) sham exposure or exposure to (B) 1 Gy, (C) 3.5 Gy, or (D) 6.5 or 8.5 Gy. Data are normalized to initial body weights and are given as mean $\pm$ SEM ($n = 4$ to 6 animals per dose cohort).

current study, doses of 6.5 and 8.5 Gy in the context of minimal supportive care led to death in the irradiated NHP, which we attributed to severe thrombocytopenia resulting in internal hemorrhage, consistent with the hematopoietic ARS mode of death.

The changes in hematology values in our study were consistent with the time course and severity scores for the ARS within each irradiation dose group. With regard to NHP that received 1.0 Gy of irradiation, almost all values for lymphocytes, neutrophils, platelets, Hgb, and Hct remained within the 95% CI recorded for nonirradiated animals for the entire 60 d, except for the immediate classic rise in absolute neutrophil count and a slight decrease in platelets below the normal range at day 18 (Figure 2). Therefore the majority of these animals had an ARS severity score of 0, with a few scoring as 1, throughout the experiment. In the other irradiated groups, scores for animals irradiated with 3.5 Gy were mainly a mixture of 1 and 2, and all NHP in both the 6.5- and 8.5-Gy groups received hematologic ARS severity scores of 3 or 4.

Our range of normal values for Hgb was similar to that reported for another minimal-support model of radiation in NHP.⁶⁹ In addition, neither Hgb nor Hct varied over 90 d after 1.0-Gy irradiation of *Cebus paella*.²² These results are consistent with ours at 1.0 Gy, after which rhesus macaques received a severity score of 0. In contrast, rodents show well-documented dose- and time-dependent decreases in Hgb and Hct, which eventually can recover to baseline values.^{55,56,67,71,72} We therefore expected and observed similar dose-dependent decreases in these hematologic parameters in our macaques: after 3.5 Gy, ARS scores were 1 or 2, and after 6.5 or 8.5 Gy, scores were 3 or 4.

The gastrointestinal subsyndrome of the ARS demonstrated strong concordance between the ARS severity score and the incidences (percentages) of emesis, diarrhea, and inappetence (Figure 4). The emetic response to ionizing radiation is well-documented to occur within the first few hours after irradiation, and diarrhea can occur at later times postirradiation. It seemed unusual that one NHP also vomited on the second day after irradiation, as this effect is not typically observed in any vomiting species. Although it might seem of interest that diarrhea occurred on day 4 after irradiation in the 2 high-dose cohorts in the current study, in another study of irradiated NHP (that received medical management), moderate diarrhea (severity score, 2) began 4 and 8 d after irradiation in 62.5% of the animals receiving 7.2 to 8.9 Gy of 6-MV LINAC-derived photons.²⁴ In our study, the severity score for diarrhea averaged between 1 and 2 for both high-dose cohorts. The diarrhea severity score in our study was identical with that of a previous study.²⁴ Whether diarrhea would have developed the remaining 2 NHP in our 6.5-Gy group is unknown. Once diarrhea began in the first 4 NHP, the veterinarian on duty prescribed loperamide for all of the animals in the group. Because the diarrhea was treatable, nonrecurring, and did not contain blood strongly suggests that these episodes were not part of the gastrointestinal syndrome. The severity of scores for each of the 3 gastrointestinal parameters increased with increasing irradiation dose (Figure 4). However, the episodes of emesis and diarrhea were transient; only inappetence was sustained.

In most species, diminished food and water intake occur in a dose-dependent manner after ionizing irradiation, but this response is immediate rather than delayed and likely is associated with deterioration of clinical signs, such as we observed in the current study. That the body weights of our NHP remained close to both baseline values and those of the sham-irradiated (control) group and that body weight change had a severity score of 0 (Figure 5) further indicate the animals were not close to death due to gastrointestinal syndrome, even though their maximal gastrointestinal ARS severity scores were 2 to 3 or 4. If these animals had been provided full medical supportive care and thus lived longer than 11 to 18 d, they might later have lost

Figure 6. Serum osmolality in an NHP radiation dose-response model. (A) Control values were determined by using preirradiation blood samples from all animals ($n = 30$) plus all blood draws from sham-exposed animals until 60 d. Frequency distributions for control values of serum osmolality fit a log-normal distribution. Time course changes in serum osmolality after (B and C) sham exposure (filled circles) or exposure to 1.0 Gy (open triangles), (B) 3.5 Gy (filled squares), or (C) 6.5 Gy (open circles) or 8.5 Gy (filled triangles). Data are given as mean $\pm$ SEM ($n = 6$ animals per dose cohort).

significant weight. The lack of decreased body weight after irradiation with 6.5 or 8.5 Gy contrasts somewhat to markedly with the results of studies involving full medical management.^{4,24} In one of these studies, the NHP rapidly lost approximately 7.5% of their average body weight within 5 d after irradiation and remained at this weight level for 30 d; in the other, 45.8% of the

NHP lost 15% or more body weight shortly after irradiation doses of 7.2 to 8.9 Gy, doses that would have been lethal in our current study.^{4,24} Even though our NHP did not lose weight, given the differences among results of this severity measure across studies, body weight should be retained as a parameter in an ARS severity score sheet.

In addition, we evaluated hydration status in our study by measuring serum osmolality. In mammals, including NHP, serum osmolality has a 'set point' at about 300 mOsm/kg.¹⁵ However, depending on the species, the mean value can range from 279 mOsm/kg (bovine) to 320 mOsm/kg (feline). Under controlled conditions, serum osmolality in NHP changes very rapidly in response to manipulation of water intake or intravenous infusion of various liquids.^{52,75,76} Furthermore, osmolality is inversely correlated with weight changes.⁷⁶ Given that our NHP had free access to both food and water and that their body weights fluctuated at different time points, these indices likely influenced the serum osmolality at blood-collection time points. In addition, blood was drawn at various intervals throughout the study. For the first 5 d after irradiation (or sham), the blood draws were timed relative to when the radiation dose was administered (for example, 4 h afterward). Thereafter, blood was drawn in random order at 0800 to 1600. This variability in sampling likely contributed to the range of serum osmolality values we obtained.

With regard to the neurovascular system, NHP that received less than 6.5 Gy showed only isolated and brief episodes of poor responsiveness. Most of these events occurred either immediately after irradiation or at a time interval that was unassociated with any noteworthy event. Conversely, NHP that received the 2 higher irradiation doses displayed significant lack of responsiveness that likely was related to multiorgan failure within 11 to 14 d after irradiation. Body temperature varied considerably within and across NHP, regardless of whether they were irradiated. Unlike in previous studies,^{43,44} we noted no radiation-dose-dependent rise in body temperature. The reason for the variation in body temperature in our study is unclear but is likely to be related to the animal's level of activity or excitement. Certainly, many other authors have reported very stable temperature values in NHP over prolonged time periods or continuously for 24 h, during which body temperatures hovered within 1 °C of normal.^{29,38,68,70}

The average respiratory rates in our study are consistent with the ranges determined through plethysmography for unrestrained cynomolgus monkeys and through telemetry for rhesus macaques.^{17,40} In the current study, the only significant cutaneous symptom was bleeding of the gums in a single NHP, which was irradiated at 8.5Gy, and the bleeding lessened in severity over a few days; this animal was euthanized shortly afterward. In summary, for organ systems other than the hematologic and gastrointestinal systems, only the parameter 'response to stimuli' in the neurovascular system showed a strong link in our ARS scoring system that paralleled that in the human scoring system.²⁶ In this case, the link (and others) was tied to animal euthanasia or end of life.

Numerous organ- and radiation-injury-specific biomarkers have been described, some of which were evaluated from the NHP used in this study.^{9,11,42,58,59,60-62} For the hematopoietic syndrome, favored biomarker candidates include Flt3 ligand, serum amyloid A, C-reactive protein, and γ -H2AX.^{5,50,59,61,62} For the gastrointestinal syndrome, citrulline is favored, although fatty acid binding protein also shows promise;⁴⁵⁻⁴⁷ these 2 markers are indicative of lost or dysfunctional small intestine enterocytes. As these cells are damaged, citrulline production is

reduced, and fatty acid binding protein is released; the increase in serum fatty acid binding protein appears much sooner than the decrease in serum citrulline.

Two other NHP studies have combined behavioral and clinical monitoring with a scoring system. Similar to our current study, one of these other NHP studies assessed basic hematology and chemistry panels concurrently with clinical behavioral signs to monitor NHP treated with streptozotocin.^{35,36} In the other study, fecal cortisol and immunoreactive cortisol metabolites and behaviors were recorded to evaluate the response to stress-associated construction work.⁷⁴ Herein, we have adapted and applied a human severity scoring model to NHP. In addition, the combined ARS assessment criteria allowed us to identify NHP (that is, those that received the 2 higher irradiation doses) for enhanced monitoring so that they might be euthanized prior to experiencing excessive pain and distress. Of the 12 NHP in these 2 dose groups, only one animal was found dead without showing any signs or symptoms that met the criteria for euthanasia.

The NHP ARS severity scoring sheet we developed here was used successfully in a follow-on study investigating radioresponses using minimum and full supportive care with and without G-CSF treatment.⁵⁷ Algorithms based primarily on the hematology and blood chemistry parameters have been developed and used to predict the severity of individual animals' overall hematopoietic ARS, reported as radiation risk and injury categorization, in both minipigs and NHP.^{13,14} These algorithms provide useful tools to merge multiple parameters into a single severity scoring scale that can be used to identify animals requiring enhanced monitoring.

Future studies should include more animals and broader dose ranges and gradients. In addition, follow-up experiments should incorporate more comprehensive measurements of the clinical status of animals, possibly including the use of noninvasive functional respiratory evaluation, measurements of multiple candidate biomarkers of different organ system injuries, and complete histologic investigations, to gauge the severity of multiple-organ injuries.⁴⁰ The proposed NHP scoring system can and should be used after exposure to different qualities and dose rates of both total-body and partial-body irradiation.

In summary, the METREPOL system has been widely adopted for use in Europe. Here we have adapted this approach and system as a refinement for the care of irradiated NHP by enhanced monitoring.^{20,26-28,34,64} Such a refinement was suggested previously¹⁶ and was consistent with published guidelines.⁶³ As we have shown in the current study, the ARS severity scoring system for NHP is a dynamic instrument that can be used to include not only the measures suggested here but accommodates exploration of other potential measurement tools to support medical management of radiation-induced multiorgan involvement and failure.⁵¹

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