

# Citrulline, A Potential Biomarker of Radiation-Induced Small Intestine Damage

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## Abstract

Radiation damage assessment of the small intestine is important in nuclear accidents or routine radiotherapy of abdominal tumors. This article reviews the clinical symptoms and molecular mechanisms of radiation-induced small intestinal damage and summarizes recent research on biomarkers of such damage. Citrulline is the most promising biomarker for the evaluation of radiation-induced small intestinal damage caused by radiotherapy and nuclear accidents. This article also summarizes the factors influencing plasma citrulline measurement investigated in the latest research, as well as new findings on the concentration of citrulline in saliva and urine after different types of radiation.

## Keywords

biomarker, small intestine damage, citrulline, radiation

## Introduction

Nuclear weapons used in wars, accidents at industrial and nuclear power plants, accidental exposures from medical sources of radiation, environmental factors, and nuclear terrorist attacks are some of the ways humans face radiation-related injuries. Where a radiation source is known, every effort should be made to avoid exposure to radiation and shorten the exposure time, avoid the radiation source, and seek shelter. Once the human body is exposed to radiation, appropriate screening and medical aid should be applied immediately. In the various scenarios mentioned above, because of differences in factors such as gender, age, radiation dose and rate, radiation type, external or internal exposure, health background, lifestyle habits, etc. identifying the irradiated population and applying timely medical assistance are very complex tasks. A radiation biomarker (a biomarker) refers to a class of substances that can be utilized to indicating the interaction between biological systems and radiation. The ideal radiation biomarker can reflect the radiation dose received by the individual, type of radiation, exposure time, damaged organ, and pathological stage of damage. Radiation biomarkers can be used to screen individuals exposed to radiation during a nuclear accident, assess health risks, and provide timely mitigation or preventive measures.

In recent decades, research on radiation markers has become increasingly detailed. Candidate genes for various radiation markers screened from human blood have been systematically

reviewed by Lacombe.<sup>1</sup> However, because the sampling and research methods of many studies are different, further research is required. The gastrointestinal (GI) tract consists of radiation-sensitive organs and it is the first to have a systemic crisis after being exposed to radiation, which affects nutrition intake and even causes systemic inflammation. Gut health assessment should be given high attention in the early stages of assisting irradiated persons. In addition, in the radiotherapy of celiac tumors, the intestinal toxicity caused by irradiation is the biggest cause of normal tissue damage and limit radiation

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**Table 1.** The Influence of Time Course and Dose on the Observation of the Severity of Small Bowel Injury Caused by Radiation in Non-Human Primates.

Species (radiation condition)	Dose	Time course	symptom
Rhesus macaque <sup>12</sup> (X-ray; Total body irradiation)	10.0 ~ 14.0 Gy	Day 4 post-TBI on average, peaking at day 7	Diarrhea
	10.0Gy	Day 5 on average, peaking at day 7	Grade 2 Dehydration
	14.0 Gy	Peaking at day 7	Grade 3 Dehydration
	10.0 ~ 14.0 Gy	Day 7 on average	Crypt numbers reaching 10% loss of pre-irradiation body weight.
	10.0Gy	On day 7 post-TBI	Crypt numbers decreased to 30% of control.
	10.0Gy	On day 10 post-TBI	Crypt numbers decreased to 24.8% of control.
	10.0Gy	On day 15 post-TBI	Crypt numbers restored to 44% of control.
	11.5Gy	On day 7 post-TBI	Crypt numbers decreased to 6% of control.
	11.5Gy	On day 10 post-TBI	Crypt numbers restored to 28.8% of control.
	11.5Gy	On day 15 post-TBI	Crypt numbers restored to 25% of control.
Rhesus monkeys <sup>13</sup> (X-ray; Total abdominal irradiation)	9.5Gy	Appeared initially at 5 days, continued through 14 days	Diarrhea
	9.5Gy	At 7 and 14 days after post TAI, but not at 35 days after TAI	Crypts/millimeter were reduced significantly relative to baseline controls.

efficiency dose. Therefore, it is necessary to pay attention to the evaluation of intestinal radiation damage in daily radiotherapy practice. This article reviews the advances in the discovery and evaluation of biomarkers of small intestine damage caused by ionizing irradiation and provides a novel review of plasma citrulline research that is most promising for clinical application.

### Radiation Damage of the Small Intestine

The intestines are the foci of radiation protection. The GI tract is a normal entrance and absorption pathway for nutrients, has a high degree of structural complexity, and constituted from multiple cell types, each performing a different function. It is fundamentally sensitive to a variety of pathogenic microorganisms, chemical, and radiation attacks. Studies have found that there are many factors that affect the initial radiation dose of small bowel injury. First, different types of radiation cause different degrees of intestinal damage. For example, neutron radiation causes more serious intestinal damage than gamma photon radiation.<sup>2</sup> Secondly, the volume of the small intestine irradiated is also an important factor, the larger the exposure volume, the more serious the damage is.<sup>3-5</sup> Irradiated volume of the small intestine affects not only acute small intestine injury but also chronic damage. Emami et al<sup>6</sup> evaluated the radiation dose associated with delayed toxicity of the small intestine. When one third of the small intestine is irradiated, the TD5/5 (5% chance of injury showing up over the next 5 years) and TD50/5 (50% chance of injury showing up over the next 5 years) were estimated to be 50 Gy and 60 Gy, respectively. While the TD5/5 and TD50/5 of whole organ irradiation were 40 Gy and 55 Gy. In addition, the fixed parts of the small intestine (such as the duodenum and the terminal ileum) are more sensitive to radiation.<sup>7</sup> Because the growth of small intestinal epithelial cells has a circadian rhythm, the time period of exposure during the day can also affect the severity of the injury.<sup>8</sup> These uncertainties make it impossible to have a clear threshold for radiation damage to the small intestine.

Acute radiation damage occurs when the systemic dose exceeds 2 Gy, and as the level of radiation exposure increases, the severity of symptoms also increases.<sup>9</sup> Studies have also found that doses as low as 1.5 Gy can cause the prodromal stage of nausea, vomiting, and gastric cancer.<sup>2</sup> In some experiments the dose that caused the collapse of the GI system was 6-10 Gy.<sup>10</sup> While some researchers believe that the losing of intestinal crypt cells and breakdown of the mucosal barrier occurs between 5-12Gy.<sup>11</sup> Moreover, from the experimental data of the monkeys listed in Table 1, it can be seen that if different observation time points are selected, different conclusions will be obtained.

The pathophysiological mechanism of gastric syndrome caused by radiation is complex, involving the loss of crypt cells, reduction in the number of intestinal villi, poor regeneration of intestinal stem cells after irradiation, and systemic inflammatory response syndrome (SIRS) caused by a variety of cytokines and growth factors.<sup>14,15</sup> In addition to the intestinal damage caused by the direct effect of radiation, it is generally believed that the bystander effect caused by radiation will also cause intestinal damage<sup>16</sup> through 2 pathways of intercellular gap and paracrine.<sup>17</sup> Some irradiated cells can cause damage to neighboring cells through soluble components<sup>18,19</sup> or release of exosomes. In 2007, Gaugler's research demonstrated that in high-dose radiation in vitro experiments, irradiated EC epithelial cells plays essential role in the initiation of the pathogenesis of intestinal damage to radiation, ie, epithelial cell lethality. A similar phenomenon was also discovered in the study when using Dark Agouti rats for fractional exposure experiments.<sup>20</sup> In recent years, the rise of organoids has led to new methods for the study of bystander effects. Enteroids, small intestinal crypt organoids, consist of a 3D epithelial monolayer that maintains crypt-villus architecture with replicating ISC intestinal stem cells that differentiated into the major small intestinal epithelial lineages.<sup>21,22</sup> Using Enteroids, Leonetti's research<sup>23</sup> have found Ceramide and its related enzyme acid sphingomyelinase (ASM) are secreted by

irradiated endothelial cells and act as bystander factors to enhance the radiotoxicity of intestinal epithelium. The rapid turnover of intestinal epithelial cells results in the intestinal mucosa being particularly sensitive to high radiation exposure during radiation therapy or any other nuclear exposure. Therefore, maintaining intestinal homeostasis is essential in order to resist radiation-induced GI damage.<sup>24</sup> After being exposed to radiation, living organisms often show active or passive changes in biological macromolecules such as nucleic acids, proteins, and metabolites in cells, organs, and body fluids. We wish those biological macromolecules can reflect the exposure time and radiation characteristics (type, dose, dose rate, etc.), and changes in biological macromolecules in such as injured organs can provide urgently required information to emergency medical service professionals.

### *Clinical Symptoms of Small Intestinal Damage Caused by Radiation*

The small intestinal epithelial cells are constantly renewed, and the cells migrate from the intestinal crypt along the sides of the villi and eventually fall off at the top. Controlling cell adhesion during cell migration, division, and differentiation is essential to maintain its healthy and sustainable regeneration.<sup>25</sup> Complex gene expression networks<sup>26,27</sup> control the steady-state of multicellular proliferation, starting from stem cells.<sup>28</sup> These overly complex control networks are most vulnerable to radiation damage.<sup>29</sup>

Radiotherapy of abdominal and pelvic malignancies usually causes severe intestinal toxicity,<sup>30</sup> which is an important clinical problem that restricts the dose determination in radiotherapy. The total risk of this complication depends on the stage of cancer, patient age, GI condition, and radiation type, dose and fractionation.<sup>31</sup> Radiation destroys and depletes stem and immature cells, making it impossible for the body to fully compensate for defects caused by the exfoliation of differentiated cells. This results in a change in the morphology of the mucous membrane in an inflamed form. In turn, the rapid natural renewal of the intestinal mucosa makes these cells particularly vulnerable to cytotoxicity treatment.

Mucositis, also known as mucosal barrier damage, has complex pathological and clinical manifestations.<sup>32</sup> It is characterized by physiological changes in the epithelial layer—from erythema to ulcers. Mucositis is also one of the most debilitating side effects of radiotherapy and chemotherapy.<sup>33</sup> The epithelial barrier lining the GI tract is composed of a single layer of epithelial cells,<sup>34</sup> forming a mechanical barrier that separates the inside of the human body from the outside world. Mucosal damage disrupts the body's natural barrier against infection. In addition, a weakened immune system is a factor leading to the dynamic development of infection. Inflammation, the loss of mucosal integrity, and neutropenia increase the risk of local bacterial, fungal, and viral infections.

### *Molecular Mechanism of Small Intestine Damage Caused by Radiation*

Owing to the particularity of the small intestine, there is currently no molecular model for small intestinal mucosal damage caused by radiation, but it is generally believed that the damage process of oral epithelial cells should be consistent with that of small intestinal mucosal epithelial cells.<sup>35</sup> Treister and Sonis<sup>36</sup> observed that the general cellular process of mucosal injury involves not only the damage to epithelial cells, but also the participation of other molecular processes. Recent studies have shown that the mechanisms involved in the pathogenesis of mucositis are more complex than direct damage to the epithelium alone. Radiation therapy with multiple doses of radiation will trigger a series of biological events in the intestinal villi epithelial cells. It is generally believed that the mucosal damage caused by radiation can be divided into 5 stages, according to the model introduced by Sonis.<sup>37,38</sup> Different regions of the mucosa may undergo each stage of damage independently.<sup>37,39</sup>

- a. Initial stage: Radiation directly damages epithelial cells, the basement membrane, and submucosal blood vessels. The direct effects of radiation cause DNA damage and the death of epithelial cells. The reactive oxygen species generated by the indirect effects of radiation are also considered to play an important role in the occurrence of mucosal damage.<sup>40</sup> The formation of these lesions leads to the activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B).<sup>41</sup>
- b. Inflammatory factor stage: This stage involves the activation of inflammatory cytokines such as interleukin 1, tumor necrosis factor alpha (TNF- $\alpha$ ), and interferons (IFN), and the initiation of angiogenesis. During epithelial cell injury and death, the second messenger is activated, leading to the upregulation of pro-inflammatory cytokines and tissue damage.<sup>42</sup> The activation of messenger molecules causes the onset of inflammation. Intestinal changes at this stage include intestinal epithelial cell apoptosis and morphological changes of the small intestinal villi.
- c. Signaling and amplification stage: This stage involves the enhanced release of cytokines, leading to mucosal damage and loss of its integrity and continuity. Primarily, macrophages begin to produce pro-inflammatory cytokines such as TNF- $\alpha$ , and activate molecular pathways that amplify mucosal damage. The cascading effect of inflammatory factors leads to the increased involvement of immune cells and apoptosis of mucosal epithelial cells.
- d. Barrier dysfunction stage: At this stage, the small intestine produces mucosal ulcers. The ulcer phase is characterized by the disruption of the continuity of the epithelial barrier. The disruption of barrier function is the result of the combined action of epithelial cell apoptosis, the development of mucosal ulcers, inflammatory cell infiltration, dysfunction of the local

immune response mechanism, and microbial translocation (viz., of bacteria, viruses, and fungi). Metabolites of intestinal microorganisms are also one of the causes of inflammatory cell infiltration.

- e. Recovery stage: Owing to the continuous differentiation and proliferation of mucosal cells, the integrity and continuity of the epithelium and the normal functioning of the small intestine villi are restored.<sup>43</sup> At this stage, the microvessels in the villi of the small intestine are also recovered further.<sup>44</sup>

### ***Biomarkers of Radiation Damage in the Small Intestine***

In recent decades, biomarkers of radiation damage in the small intestine have been researched extensively, and many methods and candidate biomarkers have emerged. Studies on the small intestine generally focus on the unique features of the small intestine, such as absorption, barriers, and amino acid synthesis. Owing to technological progress, various advanced methods such as mass spectrometry and nucleic acid sequencing have been incorporated in the research methods in recent years. The use of various omics methods has promoted the birth of more noninvasive methods. However, there is currently no small intestinal radiation damage biomarker approved by competent authorities, and there is not even a “gold standard” in the industry. Additionally, among the several possible candidate biomarkers currently under investigation, almost all of them fail to meet the screening requirements of ideal radiation biomarkers specific for radiation types. Only the radiation dose has a good correlation within some candidate biomarkers. Many potential radiation biomarkers targeting the small intestine have been proposed, such as diamine oxidase (DAO) calprotectin and gut flora. DAO<sup>45-48</sup> is a highly active intracellular enzyme in the upper villi of the small intestine of humans and mammals. It is closely related to the integrity and damage of the intestinal mechanical barrier. However, the low level of DAO in the blood makes it difficult to detect, and it is easily confused with heparin in the blood.<sup>49,50</sup> Calprotectin<sup>51,52</sup> is a calcium- and zinc-binding protein with a molecular weight of 36 kD. The concentration of calprotectin in feces has been identified as a sensitive biomarker for intestinal inflammation. It is highly sensitive and noninvasive, but low in specificity and cannot distinguish the anatomical site of intestinal injury.<sup>53,54</sup> The composition of the microbiome in gastrointestinal tract is unique to an individual. However, it is not fixed and can be altered according to various factors such as changes in environment, drugs, and diseases. Studies have shown that radiation can cause significant changes in the gut microbiota.<sup>55,56</sup> And microbiome plays an important role in the pathogenesis of radiation-induced intestinal damage.<sup>57</sup> Although compared with the sham irradiated control, the intestinal microbiome of radiated one shows a reduction of specific flora,<sup>58,59</sup> the amount of microbe reduction cannot linearly indicate the radiation dose. Moreover, certain pre-existing pathology can also affect the specificity of intestinal flora as a marker of radiation

damage.<sup>60</sup> The data from patients that scheduled to receive abdominal radiotherapy in 3 different clinical trials,<sup>61-63</sup> and normal C57 mice in an abdominal irradiation experiment<sup>60</sup> shown that the microbiota profile changed greatly before and after irradiation. However, there were discrepancies regarding the nature of these alterations between studies.<sup>64</sup>

### ***Citrulline as Biomarkers of Radiation Damage in the Small Intestine***

***Features of citrulline secretion in the small intestine.*** Citrulline is currently the most in-depth researched candidate that meets most requirements (tissue specificity, volume-response relationship, etc.). The citrulline test mainly evaluates the loss of intestinal epithelial cells, which is an important manifestation of acute and chronic intestinal radiation damage. There are currently 2 pathways found in the synthesis of citrulline in vivo, and these 2 pathways are mainly completed in the small intestine. The first is the synthesis of citrulline from glutamine, which requires 5 mitochondrial enzymes: phosphate-dependent glutaminase (PDG), pyrroline-5-carboxylic acid synthase (P5CS), ornithine aminotransferase (OAT), Ornithine carbamyltransferase (OCT), and Carbamoyl phosphate synthase I (CPSI), of which P5CS is the key regulator<sup>65-68</sup> and unique to small intestinal epithelial cells.<sup>66,69,70</sup> Proline synthesis of citrulline and arginine is another important pathway for citrulline synthesis. This pathway involves 4 mitochondrial enzymes,<sup>71</sup> namely proline oxidase (PROox), OAT, OCT, and CPSI. Although the key regulatory enzymes of this pathway, PROox and CPSI,<sup>65,72</sup> are also found in the liver and kidneys, the activity of PROox in the small intestine is relatively high, that is, 10 times and 6 times higher than that in liver and kidney, respectively, in piglets,<sup>71</sup> and the total number of small intestinal cells is much larger than liver and kidney cells.<sup>73</sup> The main consumer of citrulline is the kidney. In a study by Windmueller et al.,<sup>74</sup> citrulline utilization was measured in isolated livers perfused for 150 min with blood-plus-plasma. A tracer dose of L-[carbamoyl-14C] citrulline was added to the recycling perfusate, which contained 124  $\mu$ M citrulline. After 150 min and about 40 passes through, about 90% of the labeled citrulline remained, which indicates that the liver is very inefficient in metabolizing citrulline. In contrast, 35% of citrulline is consumed by arterial blood as it passes through the kidney.<sup>74,75</sup> Nowadays, the small intestinal absorptive epithelium is widely regarded as the main source of circulating citrulline.

In several organ exclusion experiments, it was observed that no part of the body, except the intestine, releases large amounts of citrulline under physiological conditions.<sup>74</sup> The use of specific inhibitors of small intestinal OAT<sup>66</sup> and OCT<sup>76</sup> for small bowel targeting intervention significantly reduced plasma citrulline concentrations, which can also support this conclusion.

Experimental and clinical data<sup>77</sup> have shown an uneven distribution of citrulline production within the small intestine. It was observed that the P5CS activity of rats is distributed in the duodenum, upper jejunum, lower jejunum, and ileum at 26%, 31%, 33%, and 10%, respectively.<sup>69</sup> However, the data

provided by Crenn et al.<sup>78,79</sup> indicate that there is a volume effect. The decrease in intestinal absorption after irradiation is associated with the loss of functionally active intestinal epithelial cells that make up the surface of the absorbable mucosa.<sup>80-83</sup>

**Plasma citrulline concentration and the radiation dose.** The relationship between the plasma citrulline concentration and the radiation dose was proven many times through experiments and clinical studies. Lutgens et al<sup>84</sup> used female NMRI mice to investigate the relationship between plasma citrulline levels and X-ray-induced small intestine epithelial cell loss and small intestinal morphology. The conclusion is that the plasma citrulline concentration changes most significantly at the time points of 84 hours and 4 days after IR. At low doses (0-3 Gy) the plasma citrulline concentration changes, although it is not obvious, but at a high dose (3-12 Gy), the decline is obvious. After the fourth day, the citrulline level began to recover and reached normal level in mice that received less than 8 Gy irradiation, while mice that received higher doses of irradiation were not able to fully recover. Lutgens et al<sup>85</sup> conducted a prospective clinical study in patients undergoing graded radiotherapy for abdominal and/or pelvic cancer sites (23 patients, 9 males, 14 females, 28.3-72.6 years old). After the initiation of radiotherapy, the citrulline concentration showed a decrease in relation to the dose received and the volume of the intestine. The citrulline concentration in the last 3 weeks of treatment showed correlation with evaluated clinical toxicity. The acid concentration showed more relevant correlation with the dose or exposure volume than with the evaluated clinical toxicity. From November 2008 to May 2010, 53 patients (36 prostate cancer, 17 endometrial cancer) who underwent pelvic radiation therapy were prospectively reviewed in Turkey.<sup>30</sup> A strong correlation between dose-volume and citrulline concentration was also observed, and the authors recommend that citrulline concentration should be included as an indicator of intestinal toxicity caused by radiation in future clinical practice. The relationship between citrulline concentration and intestinal epithelial cell loss is also observed in other pathological conditions not related to radiation, such as surgery after small bowel transplantation,<sup>78,79,86,87</sup> celiac disease and non-Celiac disease,<sup>88</sup> and viral enteritis.<sup>89</sup> Overall, plasma citrulline appears to be a quantitative parameter and it is not depend on related to the underlying cause of epithelial cell loss.<sup>90</sup>

### **Non-Radiation Factors Affecting Plasma Citrulline Concentration**

Crenn<sup>91</sup> examined several non-IR factors that affect plasma citrulline concentrations, including diet, age and ethnicity, renal function, metabolic stress and inflammation, and liver function. Recently, a study using 3 types of animals (mice, minipigs, and Rhesus macaques)<sup>92</sup> found that the citrulline level was significantly reduced by 35.5% ( $P < 0.0017$ ), when nonhuman primates (NHPs) anesthetized with ketamine and acepromazine compared with unanesthetized NHPs. It is also

found that in the postprandial state, the concentration of citrulline in NHPs decreased slightly, but decreased significantly by 12.2%. These results indicate that plasma citrulline is affected by experimental conditions such as anesthesia and feeding. In a study by Park et al<sup>93</sup> it was found that serum citrulline levels in mice showed diurnal changes and fluctuations related to food intake with no significant simultaneous change in the intestinal cell mass. Serum citrulline levels in fed mice did not change daily, while in fasted mice it was significantly higher in the morning than at night. These findings highlight the importance of consistency in sample collection strategies in translational research.

### **Citrulline in Non-Plasma Body Fluids**

Because blood extraction can cause damage to the body, blood extraction and storage require professional skills; thus, it is not the most suitable method for large-scale screening of radiation-exposed individuals. In addition to detecting citrulline in plasma, changes in citrulline concentrations have also been detected in urine and saliva after exposure to radiation. In an experiment in which 3 male and 4 female rhesus monkeys (*Macaca mulatta*) were irradiated with cobalt 60<sup>94</sup> at the dose of 4 Gy, saliva was collected at different time points and citrulline levels were determined by an ultra-high performance liquid chromatography system in combination with Xevo G2-S time-of-flight mass spectroscopy (TOF-MS). The results showed that on the first day after irradiation, the citrulline in saliva increased rapidly to more than 2 times of that before the irradiation, and then rapidly decreased again on the third day. This result is the exact opposite of how plasma citrulline responds to radiation (which drops significantly in blood). In C57BL/6 mice, the  $\gamma$ -ray (cesium 137) irradiation dose was 0.5-8 Gy, and the sampling time was 1 and 7 days after irradiation. No change in citrulline concentration was found in saliva.<sup>95</sup> Although saliva is easy to obtain, as a biomarker its application has many restrictions because of factors such as smoking,<sup>96</sup> circadian rhythm,<sup>97</sup> eating habits,<sup>98</sup> and so on. In some pathological situations, saliva contains certain blood components, which may affects the results.<sup>99</sup>

Goudarzi and colleagues<sup>100</sup> applied different radiation patterns to C57BL/6 mice, and compared the effects of internal (Sr 90 and Cs 137) and external irradiation (low and high dose rates of X-rays). The results showed that there was no statistically significant change in urinary citrulline concentration within 24 hours after 4.4 Gy irradiation with X-rays at a low dosing rate (3.0 mGy/min). The citrulline levels in mice were significantly reduced after 90 days of 90Sr (internal irradiation) exposure with a cumulative dose of 2.0 Gy, and Cs 137 (internal irradiation) on the fifth day after exposure with a cumulative dose of 4.1 Gy also showed a similar trend. After X-ray irradiation with 4.4 Gy at a high dosing rate (1.1 Gy/min), the citrulline level on the 5th day increased significantly. The results of this study are very important. It illustrates that changes in citrulline can be detected under internal radiation, and inhalation internal radiation occurs in many nuclear

accidents. In several other investigations involving nontargeted mass spectrometry detection of sources other than irradiation, the change in citrulline was not detected in radiation-exposed mice,<sup>101</sup> rats,<sup>102</sup> and monkeys.<sup>103</sup> This may be due to the nontargeted approach used. It can be concluded from the above researches that the level of citrulline in body fluid can directly represent neither the change of citrulline concentration in plasma nor the loss of small intestinal epithelial cells.

## Conclusions

There are different types of radiation in various scenarios, and they affect different groups of people (classified based on protection level, gender, type of radiation exposure, age, education level, etc.). In the case of a certain group size, appropriate measures should be taken. This calls for a targeted approach, which can combine clinical symptoms and biomarkers to achieve the current optimal solution. The currently used methods aim to find a marker that meets all conditions. However, the reality is that in different biological processes, biomolecules that can play a role in marking are often different. Thus different biomarkers should be used in different biological and clinical symptom stages, and radiation-induced tissue damage cannot be expressed or quantified by a single functional or morphological parameter.<sup>104</sup>

In addition, as blood citrulline is being increasingly accepted for its high-dose external radiation, clinicians are hopeful that the concentration of citrulline can be consistent with the current clinical toxicity classification system. If clinical toxicity classification and radiation biomarkers can be mapped with clear biological processes and clinical symptoms, it will be greatly beneficial to the decision-making process.

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
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