The latest findings of gamma irradiation of blood products to prevent transfusion-associated graft-versus-host disease (TA-GVHD) in cancer patients: A systematic review

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ABSTRACT

Blood products including RBC, platelets and plasma are used for the treatment of different diseases, especially in cancer patients. Administration of these derivatives may be associated with a wide range of adverse effects. Transfusion-associated graft-versus-host disease (TA-GVHD) is a fatal complication resulting from blood transfusion. Currently, irradiation of blood products containing cells, which can be achieved using X-ray or gamma ray, represents an optimal approach to prevent TA-GVHD. To the best of our knowledge, this is the first systematic review to address studies conducted to evaluate the effects of gamma irradiation from different sources of 60Co and ^{137}Cs on the laboratory quality of platelets (PCs) and red blood cell concentrates (RBCCs). The results of our review on pre-storage (day 0) gamma irradiation of platelet products (apheresis and PRP) for 7 days of follow-up showed that there was no significant difference between non-irradiated and pre-storage ^{137}Cs-irradiated PCs. In addition, ^{60}Co-irradiated PRP before storage also showed comparable results to their non-irradiated counterparts. Results of our retrospective study on pre-storage (day 0) gamma irradiation of red blood cell concentrates (RBCCs) products for 28 days of follow-up, demonstrated that the viability of CPDA-1-preserved RBCCs appears to

be 14 days post-irradiation, while this period for SAGM-preserved RBCCs is up to 21 days. Preservation of irradiated red blood cells in mannitol-containing solutions reduces lipid peroxidation. Overall, the results if our study showed that irradiation time and storage conditions, including preparation methods, anticoagulant/additive solutions, filtration, and washing, affect the quality of transfused blood products.

Keywords: Blood components; Gamma irradiation; Platelets; Red blood cells; Storage

1. INTRODUCTION

Platelet (PCs) and red blood cell concentrates (RBCCs) are the two most widely used blood products, which could potentially save millions of lives worldwide [1-11]. PCs are transfused to develop hemostasis in patients with thrombocytopenia or platelet dysfunction, and RBCCs are mainly used to prevent hemorrhage caused by surgery, trauma, and various malignancies or to treat patients with hemoglobinopathies and anemia [3, 7, 8, 10, 12-18]. Transfusion-associated graft-versus-host disease (TA-GVHD) is one of the most fatal complications caused by blood transfusion, in which viable T-lymphocytes from donors can attack the recipient's organs [19, 20]. Upon transfusion, helper CD4+ cells can be activated by foreign Human leukocyte antigens (HLAs), leading to the release of cytotoxic CD8+ cells [21-23]. Thus, an HLA-mismatched transfusion, whether class I or class II, can cause the immune system of an immunocompetent recipient to attack the transfused T lymphocytes [19, 24, 25]. However, if there is an HLA haploidentical match between the donor and recipient or the recipient is immunocompromised, these lymphocytes can remain proliferative and attack host tissues [19, 24]. Due to the immunodestructive response against bone marrow (BM), TA-GVHD leads to BM aplasia and severe pancytopenia, which is the main cause of death [26, 27]. Therefore, the number of proliferative lymphocytes in the blood bags, the susceptibility of the recipient's immune system, and the level of HLA disparity between the recipient and donor are factors influencing the occurrence of TA-GVHD [19, 24, 28]. Although the mortality rate of this disease is approximately 90%, there is no effective treatment for ameliorating the related symptoms. Thus, inactivation of viable lymphocytes prior to transfusion appears to be a preventive approach for TA-GVHD [29, 30]. Irradiation and leukocyte reduction filters (LRFs) can be mentioned among the most well-known methods to remove leukocytes from blood concentrates. However, irradiation has been considered as the gold standard method for leukocyte inactivation [31].

For susceptible individuals, all products containing active lymphocytes, whether prepared by whole blood (WB) or apheresis methods, should be irradiated [32, 33]. Blood irradiation can be performed using gamma ray or X-ray, both of which inactivate the proliferation of nucleated cells [34].

Gamma irradiation is an ionization electromagnetic radiation that is released through the decay of certain radioisotopes, such as ^{60}Co and ^{137}Cs [34]. It has been hypothesized that gamma irradiation may directly or indirectly damage DNA in cells. The direct effects of gamma rays can break double-strand chains of DNA and trigger the formation of random cross-linking. However, the radiolytic cleavage of water, as an indirect effect, can lead to the production of ions, free radicals, and reactive oxygen species (ROS), which in turn induce oxidative stress in blood cells. Thus, irradiation can inactivate all nucleated cells [35, 36]. Subsequently, not only can they inhibit viable T lymphocytes, but also they can alter the quality of other irradiated blood products. For example, an increase in K^+ leakage in RBCCs during storage can limit a large-volume transfusion [37-39]. In addition, an increase in ROS levels in irradiated blood bags can induce oxidation of hemoglobin

and peroxidation of lipids [40]. Thus, optimization of the irradiating conditions needs to be considered as a prime concern in blood banks. Hopefully, it is shown that all of these adverse effects are dose dependent. For instance, 15 Gray (Gy) and 50 Gy can mitigate the proliferation of T cells by approximately 87.5% and 96.75%, respectively [41-43]. In the Blood Transfusion Organization of Iran, the approved radiation dosage for blood products is 25 Gy at the center of the product bag, with a minimum of 15 Gy in other areas of the bag which is similar to regulation in the USA (approved dosage ranges from 15 to 25 Gy), while in Europe, it ranges from 20 to 30 Gy [44].

To reach such a dose, a dosimetric system must evaluate the dose rate, dose in blood containers, and dose distribution in irradiated blood volume [34].

In addition to radiation dose, various studies have shown that changes in both storage conditions and timing of irradiation can also affect the quality of products, resulting in some deleterious changes in the morphology, biochemical properties, and function of blood components [45-48]. When it comes to RBCCs, they can be irradiated from the day of collection until day 14, and can then be stored up to 14 days after irradiation (28 days in total). For hypokalemic patients, it is recommended that concentrates be transfused up to 24 h after irradiation [32]. The PCs, should be irradiated from day 1 to day 5 because of the storage time limit of 5-days [32]. Therefore, different storage conditions and timings of irradiation may cause different storage lesions of the blood products. To the best of our knowledge, there has been no comprehensive review of the effects of gamma irradiation on blood products. Therefore, the purpose of this review study is to investigate the quality of PCs and RBCCs irradiated using both ^{60}Co- and ^{137}Cs-irradiators (with doses between 15 and 30 Gy) and stored under different storage conditions. In addition, the relevant tests used to evaluate the quality of blood components and the impacts of pre-storage, post-storage, additive solutions, filtration, and washing upon the irradiated PCs and RBCCs have been explained.

2. SEARCH STRATEGY AND DATA SOURCES

A thorough review of the literature was conducted using the PubMed database. The search incorporated specific keywords, including "(RBC) AND (gamma) AND (cobalt)", "(RBC) AND (gamma) AND (cesium)", "(platelet) AND (gamma) AND (cobalt)", and "(platelet) AND (gamma) AND (cesium)". In addition, the backward and forward snowballing approach was used to identify any additional relevant research articles. Overall, 42 articles have been reviewed; 16 research articles regarding platelet gamma irradiation and 26 research articles regarding RBC gamma irradiation. Because of the lower half-life of the Cobalt irradiator, it had been used in preliminary studies; In addition to meet the outcome of this study of reviewing over 40 articles, we had to extend the period of our search from 1980 to 2023.

3. PLATELET CONCENTRATES (PCs)

3.1. Preparation of PCs

Platelet concentrates can be prepared either by whole blood (using platelet-rich plasma (PRP) and buffy-coat (BC) methods) or by apheresis approach. Furthermore, platelet concentrates may be leukoreduced either after preparation or before transfusion [47]. As a result, these processing methods can affect PCs in a distinct manner. Storing PCs at room temperature (RT) with constant agitation for 3 to 5 days is the optimal storage condition [49, 50]. The longer PCs are stored, the more functional and structural alterations, so-called platelet storage lesions (PSLs), are induced in platelets [47]. PSLs trigger the release of platelet microparticles (MPs) from the products, which

should normally be present in a small amount in blood bags [51]. After transfusion, PSLs also cause platelets to be cleared faster by the reticuloendothelial system (RES) [52].
Accordingly, a myriad of factors, including preparation methods, pH, temperature, type of blood containers, type of anticoagulants/additive solutions, platelet counts, and agitation intensity, can affect the quality of stored PCs [49, 53-56]. During blood collection, high centrifugation forces can also induce PSLs by enhancing platelet fragmentation, activation, and release of metabolic effectors [47]. Storage of PCs at room temperature can increase glycolysis and decrease mitochondrial function, resulting in glucose depletion, lactate accumulation, and acidosis [47]. The lower ATP can be generated in cells, the greater damage to cellular capacity to complete energetically demanding function [47, 57].

3.2. Factors affecting the survival of stored platelets

3.2.1. *Enviromental pH*

One of the factors affecting the survival of stored PCs is the pH of the environment [58]. Using blood bags with the ability to exchange gases is a more efficient approach to maintain the optimal pH of PCs during storage [55]. Since the oxygen permeability of the first generation of blood bags (i.e., polyvinyl chloride (PVC) plasticized with di(2-ethylhexyl) phthalate (DEHP)) is too weak, the storage time of platelets in these containers is up to 3 days [59]. However, the second generation of bags (i.e., PVC plasticized with tri(2-ethylhexyl) trimellitate and polyolefin) have a higher oxygen permeability, thus improving the storage time up to 5 days [60, 61]. Moreover, the synthesis of PVC plasticized with butyryl-tri-n-hexyl-citrate, which has a great ability to exchange gas, has enhanced the in-vivo transfusion efficacy of stored PCs [62, 63].

3.2.2. *Energy supply*

The supply of energy to the cell through ATP hydrolysis is the second most effective factor in platelet survival during storage [64]. Normally, 85% of ATP is supplied through aerobic pathways, i.e., the Krebs cycle, whereas 15% is synthesized through anaerobic pathway, i.e., glycolysis [65]. The Krebs cycle begins with the use of free fatty acids obtained from plasma or citrate added to the storage medium and ends with CO2 production [66]. The glycolysis pathway, however, starts with the hydrolysis of glucose and ends with lactate and H^+ [67]. Bicarbonate ions in storage media can neutralize these acidic substances by converting them into CO2 and H2O. Therefore, the buffering capacity of the environment can also affect platelet survival [59]. It should be noted that an increase in CO2 as a volatile acid could also affect the pH value. Thus, oxygen-permeable blood containers can participate in controlling the pH of the environment by removing CO2 driven by cellular metabolism [68].

3.2.3. *Storage media composition*

Another effective factor in preserving platelets during storage is the composition of the storage media [59]. To maintain energy production in cells, acetate as a substrate of the Krebs cycle should be added to platelet additive solution (PAS; containing acetate, citrate, sodium, and glucose) [69]. Thus, a medium containing 70% PAS-II (T-Sol) and 30% plasma can guarantee platelet preservation for up to 7 days [70]. In addition, adding phosphate to a medium called PAS-III (InterSol) increases the production of adenine nucleotides, stimulates glycolysis, and improves the buffering capacity of PAS [71]. Adding potassium and magnesium to a storage medium called PAS-IIIM (SSP+), as well as removing phosphate from it, has also been able to improve the in-vitro quality of stored platelets [72].

3.3. PLS tests for stored PCs

To evaluate the severity of PSLs, it is important to study the survival, morphology, biochemical properties, and hemostatic activity of stored platelets before and after transfusion. Tests are conducted on platelet units either on day 0 (pre-storage) or on the first-, third-, or fifth day following storage. In the following section, some of the most important tests for measuring the quality of platelet concentrates have been introduced.

3.3.1. *Metabolic and viability tests*

Because of glucose depletion, a decrease in pH can be seen in cells, which in turn induces platelet lysis [73]. Therefore, the measurement of lactate dehydrogenase as a membrane enzyme is an indicator of platelet lysis. Hypotonic shock response (HSR) is another test to evaluate platelet viability. In this regard, adding hypotonic solution to platelets induces an initial osmotic swelling of the cells, which can subsequently return to normal size after a while. The reason for this phenomenon is that the concentrations of intracellular Ca^{+2} and ATP can be increased by hypotonic shock, ultimately leading to the exit of water, K^{+} and Cl^{-} from the living cells to correct the size of the cells [74-76].

3.2.2. Morphological tests

Due to the discoid shape of platelets, they reveal the swirling phenomenon under a light source. A swirling score also indicates that the pH of the environment is in the range of 6.7–7.5 [77].

3.3.3. Functional tests

During storage, changes in the expression of platelet surface glycoproteins (GPs) and thrombin receptors can alter platelet activation. While the expressions of P-selectin (CD62P) and GPIIb/IIIa are increased during storage, GPIb expression is reduced [78, 79]. These changes in the expression of platelet GPs can accelerate the RES clearance of stored platelets [80]. GPIb and GPIX form a complex with the transmembrane form of GPV to participate in collagen and thrombin binding and provide a receptor for von Willebrand factor. The soluble form of GPV is used in platelet quality tests because it is released from activated PCs [81]. Agonist-induced platelet aggregation is another functional test that can be performed using collagen, ADP, and thrombin receptor activating peptide (TRAP) [82]. The evaluation of platelet- and leukocyte-derived cytokines can also be performed because they can participate in some allergic and non-haemolytic reactions after transfusion. Eventually, thromboelastography (TEG) and free oscillation rheometry (FOR) are techniques that can be applied to determine the quality of clot formation in both WB and PRP by checking the clotting time and the elasticity of the formed clot [83-85]. TEG depicts a graphical scheme of platelet function, coagulation properties, fibrin polymerization, and lysis. The R value indicates the amount of time required to form the initial fibrin from the beginning of the test. In addition, α angle illustrates the rate of cross-linking and fibrin formation, whereas maximum amplitude (MA) represents the maximum clot strength [84].

3.3.4. Lipid composition evaluation test

Platelet function depends on its lipomics and metabolomics because platelets lack DNA. The lipid membrane composition of platelets is altered upon activation. Moreover, during hemostasis, bioactive lipid mediators are produced to redistribute platelet phospholipids to allow coagulation factors to bind. Mass spectrometry and chromatography are used to evaluate the lipid compositions

of platelets, including free fatty acyls, glycerolipids, phospholipids, sterols, and sphingolipids. A great review on the lipid compositions of platelets in response to storage and activation was previously conducted by Green et al. [86]. Regarding metabolomics, cold-stored platelets exhibit lower oxidative stress by preserving pentose phosphate pools and glutathione. These PCs have a lower need for energy due to revealing a reduction in both amino acid catabolism and beta-oxidation markers. Moreover, the glycolysis rate is stable in both RT-stored and cold-stored PCs for up to 7 days. It is noteworthy that RT-stored PCs have a lower glucose content than cold-stored PCs. Thus, metabolomics analysis can provide invaluable information about PC quality. Details of the metabolomics alternation of stored platelets are provided by Zhao et al. [87].

3.3.5. Immune-related soluble factors

Soluble factors are stored in α- and dense granules of platelets and can be released following platelet activation. Thus, activated platelets are well-known regulators of the immune system because they can enhance leucocyte activation factors (i.e., sCD40L, RANTES, PF4, NAP2) leucocyte proliferation markers (i.e., PF4, NAP2) and inflammation markers (i.e., IL-1β, RANTES) [88].

3.3.6. In-vivo efficacy tests

Corrected count increments (CCIs) can be determined by transfusing platelets into thrombocytopenic patients. Blood transfusion can be efficient if CCIs for immediate clinical effect, which reveals platelet recovery, and late clinical effect, which indicates platelet survival, are more than 7.5 and 4.5, respectively [84, 89, 90]. However, ICC is a good marker in the context of oncohaematology but not in intensive care. An alternative method for that is radiolabelled platelets (^{51}Cr), which can measure the survival and recovery of stored platelets in healthy individuals [74]. A blood transfusion can be efficient if above 66% of the stored platelets are recovered, and above 58% of them are viable. Measuring the bleeding time can also be used to evaluate the functionality of transfused platelets [90].

3.4. Quality testing of irradiated PCs under various storage conditions

Various storage conditions and timing of radiation can induce different effects on the quality of platelet products, which are explained below (Table 1).

3.4.1. Pre-storage irradiated apheresis PCs

In a study conducted by Mallhi et al. to compare the 25 Gy gamma irradiated and non-irradiated apheresis PCs, a slight decrease in the platelet count of irradiated units was observed on day 5 of storage. Moreover, the irradiated units revealed higher CD62P expression from day 1 to day 5 of storage. During a 5-day storage period, PCs had normal morphology and swirling scores. Furthermore, the levels of Na^+, K^+, and HCO3, as well as pO2 and pCO2 in the irradiated units, were similar to the non-irradiated units. However, the irradiated units had lower glucose and higher lactate levels on the 5th day of storage. Between days 5 and 7, the irradiated concentrates showed higher lactate and lactate dehydrogenase (LDH) levels and lower glucose levels than the non-irradiated units [91].

In a similar study by Tynngard et al. between days 1 and 7 of storage, functional tests of PCs demonstrated that CD42b (GPIba) expression was well-maintained for up to 5 days, whereas P-selectin was overexpressed. After 5 days, both GPs were even more overexpressed [92].

Results from another study performing irradiation with 30 Gy on day 0 of storage showed an increase in the ADP (20 μM)-induced aggregation in the units. They also showed better ADP (10 μM)/epinephrine (2 μM)-induced aggregation on day 5 of storage [93].
Furthermore, a clinical study assessed the hemostatic function, transfusion efficacy, and safety of gamma-irradiated apheresis PCs and non-irradiated PCs in patients with chemotherapy-induced thrombocytopenia. leukoreduced PCs were split into two groups to be gamma-irradiated with 25 Gy or remain non-irradiated. There were no significant differences in angle of clotting, time to initiate clotting, and clot strength between hematologic patients receiving either irradiated or non-irradiated PCs before transfusion, as well as 1 and 24 hours after transfusion. Also, no differences were observed in terms of bleeding, adverse events, and acute transfusion reactions. The study confirms the safety of gamma-irradiated PCs for the treatment of thrombocytopenia [84].

3.4.2. Post-storage irradiated apheresis PCs

Besides pre-storage irradiation, Zimmermann et al. performed 30 Gy gamma-irradiation on days 3 and 5. The in vitro properties of irradiated platelets were comparable to those of control platelets, regardless of whether irradiation was performed on Day 3 or Day 5. However, platelets irradiated on Day 0 showed significantly better in vitro aggregability immediately after irradiation. They concluded that gamma radiation did not have any negative effects on the quality of platelets in WBC-reduced PCs. In fact, they observed a slight, but significantly better, in vitro aggregability in PCs irradiated before storage compared to platelets irradiated later during storage and unirradiated platelets [93]. Irradiation on day 3 of storage could not induce any change in HSR, P-selectin expression, ATP, pO2, pCO2, lactate, glucose, LDH, TNF-α, and IL-8 levels in the irradiated PCs compared with the non-irradiated PCs [73, 93].

3.4.3. Pre-storage irradiated PRP

A study was conducted by Zhao et al. to examine the impact of pre-storage 25 Gy gamma-ray irradiation on the expression of CD62p, the platelet count, and the mean platelet volume (MPV) in manually enriched platelet suspension. The results demonstrated that the expression level of CD62p on platelets increased over time in both the irradiated and control groups. There were no significant differences in CD62p expression rate, platelet count, and MPV between the irradiated and non-irradiated unites after 24 and 72 hours of preservation. However, the MPV of both the irradiated and control groups after 72 hours of preservation was higher than that of fresh platelets. They concluded that, gamma-ray irradiation does not impact the quantity and quality of platelets, but the preservation time for manually enriched platelet suspension should be minimized whenever possible [94].
In a similar study, ^{60}Co-irradiators were used for 25 Gy irradiation of PRP before storage. It is shown that platelet yield, MPV, platelet distribution width (PDW), and viability were similar to those of the non-irradiated units on day 2. The morphological score and HSR were comparable with those of non-irradiated units [95].

3.4.4. Pre-storage irradiated of apheresis platelets Vs. photochemically treated apheresis platelets (PCT PCs)

Photochemical treatment, which is combined of amotosalen and long-wavelength ultraviolet light, has been shown to reduce the risk of TA-GVHD by affecting all DNA-containing cells [96, 97]. Thus, this effect not only affects contaminating white blood cells (WBCs) but also induces changes in other blood cells [98]. For instance, in two similar studies both comparing PCT PCs and

irradiated PCs it was shown that storing from day 1 to day 12 could reduce the platelet yield in the pre-storage leukoreduced PCT PCs, especially after day 7, while it remained unchanged in the irradiated units. This can be due to platelet fragmentation in PCT PCs during storage, which can be demonstrated using a significant increase in LDH levels and CD61+ microparticles in these units compared with the irradiated units [51, 99]. The pH value, however, remained within the range of 6.4–7.4 for both groups. Changes in glucose and lactate levels were observed in the PCT PCs after 7 days, but in the irradiated units after 11 days. Between days 1 and 7, both CD61+ microparticles, as platelet fragmentation markers, and CD62P+ platelets, as platelet activation markers, were higher in the PCT PCs than in the irradiated units [51, 99]. After 7 days, both CD62P+ platelets and platelet-derived cytokines reached a plateau in PCT PCs [99]. The platelet-derived cytokines (i.e., β-thromboglobulin, CCL5, CXCL4, CXCL8 and TGF-β) were increased in both groups, but they were slightly higher in PCT PCs, especially after day 5. However, WBC-associated cytokines (i.e., IL-6, IL-10, IL-11, IL-12p70, IFN-γ, and TNF-α and –β) were undetectable in both groups [51, 99]. ADP/TRAP-induced CD62P expression and ADP/TRAP-induced CD42b downregulation were reduced in PCT PCs during storage, especially after 5 days. After 5 and 7 days, the number of TRAP-induced CD61+ microparticles was slightly higher in PCT PCs. Only on day 1 of storage, a significant aggregation was seen in collagen (5 mg/mL)-treated units. High initial maximum aggregation and aggregation velocity were observed in collagen (25 mg/mL)-treated units, which dramatically decreased from day 1 to day 5. PCT PCs showed lower aggregation and velocity than the irradiated units. TRAP-induced aggregation remained high until 7 days in the irradiated units, but it was steeply reduced in PCT PCs after 5 days. Both groups showed a reduction in their swirling scores [51].

3.4.5. Pre-storage irradiated apheresis PCs Vs. Mirasol riboflavin-based pathogen reduction technology (PRT) treated apheresis PCs (PRT PCs)

The Mirasol system is a pathogen reduction technology (PRTs) which combines riboflavin, as a sensitizer, with UV light to induce irreversible modification on DNA-containing cells, such as platelets and leukocytes [100, 101]. Based on a previous study, aimed to assess the impact of Mirasol PRT process on the development of platelet storage lesion, the platelet count in PRT PCs was similar to that in both irradiated and non-irradiated units [101]. The ristocetin-induced aggregation was reduced in all units during storage, but all units showed an increase in collagen-induced P-selectin, especially in the PRT PCs. Although mitochondrial membrane polarization was slightly reduced after PRT treatment, it was normal after gamma irradiation. The levels of K^+ leakage and LDH in the PRT PCs were similar to those in the irradiated and non-irradiated units. The PRT PCs exhibited higher glycolytic activity by possessing lower glucose and higher lactate levels. Moreover, PRT PCs exhibited higher oxidative respiration. On day 5 of storage, the swirling score for PRT PCs was lower than that in both irradiated and non-irradiated units [101]. Based on various proteomics analysis, the levels of red blood cell (RBC) proteins were altered in untreated apheresis PCs during storage, which was observed by reducing actin and increasing 14-3-3, gelsolin, and DJ-1 [102-105]. The Mirasol-treated PCs showed lower actin expression compared with the untreated PCs on day 1, which was consistent with the result of downregulating the nuclear chloride channel in PRT PCs [102-105]. Moreover, p47, the substrate for protein kinase C, was reduced after PRT. Although there was no significant difference between the PRT PCs and irradiated units in response to proteomic analysis, the levels of p47/pleckstrin and parvalbumin α, which are other aging markers, were altered more in PRT PCs than in their irradiated counterparts. The highest levels of these proteins were observed in the untreated PCs on day 1, and then they

were downregulated with the prolongation of storage time in all units, with the highest reduction being for PRT PCs. All of these results indicated that the PRT procedure can hasten PC aging. Storage of the irradiated PCs was either related to accelerated aging, which was supported by the downregulation of parvalbumin, LDH, and actin cytoplasmic 2-like isoform, or related to quicken aging, which was demonstrated by upregulation of 14-3-3 protein, α-actin, Ras-related protein Rap-1b isoform 1, adenine phosphoribosyl transferase, and gelsolin [105].

The DJ-1 protein, on the other hand, plays an important role in controlling the antioxidant stress in platelets. At the end of storage, this protein is overexpressed to control the significant reduction in the level of cellular glutathione (GSH) in apheresis PCs [104]. Surprisingly, DJ-1 was upregulated by irradiation [105]. However, the ER-60 protease, which is responsible for regulating the redox-induced alternation of protein disulfide bonds, was downregulated in the irradiated units more than in the untreated PCs [105, 106]. With regard to oxidative stress effectors, day 1 irradiated PCs were comparable to day 5 untreated PCs. Metabolomic analysis showed that the GSH level in day 0 irradiated PCs was as low as that in untreated PCs on day 5. In comparison with the irradiated PCs, the PRT PCs were less prone to deal with oxidative stress, which was attributed to the downregulation of some redox-sensitive proteins, including chaperone, ubiquitin, tyrosinase, and xanthine dehydrogenase/oxidase, in these units [105].

There is also a decrease in the levels of LDH and adenine phosphoribosyl transferase isoform A during storage, which makes sense when we compare these results with data on glucose consumption, lactate accumulation, and a drop in pH over this period [106, 107]. With regard to PRT PCs, it seems that these concentrates were less metabolically active compared to the untreated PCs on day 1. This was because mitochondrial ATP synthase was downregulated in PRT PCs [105]. On the other hand, gamma irradiation could induce PSLs in PCs by downregulating Rho GDP-dissociation inhibitors 1 and 2, as well as upregulating Ras-related protein Rap-1b isoform 1, which regulates integrin aIIbb3. The regulation of integrin aIIbb3 plays an important role in maintaining platelet functional activity [107].

4. RED BLOOD CELL CONCENTRATES (RBCCs)

4.1. Preparation of RBCCs

These blood concentrates can be prepared using both WB and apheresis methods. The function and morphology of RBCs should be maintained during the storage period. In this regard, RBCCs are stored in 2-6˚C for 42 days. During storage time, some morphological, biochemical, and functional changes may affect RBCCs, which are referred to as red blood cell storage lesions (RSLs) [45]. Different factors, including preparation methods, pH, temperature, type of blood containers, and type of anticoagulants/additive solutions, can affect the quality of stored RBCCs [45, 108, 109].

Red blood cells can be stored in containers made of PVC plasticized with DEHP or n-butyryl-tri(n-hexyl)-citrate (BTHC). Leaching of plasticizers inhibits hemolysis by preserving the RBC membrane. Non-PVC containers can also exchange blood gases; however, they show more hemolysis than PVC containers. Although leaching of the plasticizer DEHP can prevent hemolysis, it causes some cell deformation at the end of the storage period [59]. In addition to blood containers, anticoagulants and additive solutions play an important role in the prevention of RSLs. Acid citrate dextrose (ACD) solution, which is anticoagulated by citrate, can preserve RBCCs for up to 21 days [110]. By adding sodium phosphate to ACP, a citrate phosphate dextrose (CPD) solution is synthesized, which reduces phosphate leakage from stored RBCs by reducing the phosphate gradient between the inside and outside of the cell [46]. Next, a citrate phosphate

dextrose adenine (CPDA) solution was synthesized by adding adenine to CPD. Because RBCs lose their adenine during storage, they are unable to synthesize ATP and maintain their osmotic fragility. However, the CPDA solution can increase the storage period of RBCCs up to 35 days by supplying adenine [111, 112]. Addictive solutions with saline, adenine, and glucose, known as SAG (Saline, Adenine, Glucose), were then introduced to reduce the viscosity and storage hematocrit of RBCCs; however, hemolysis is high in this solution [113, 114]. In order to reduce hemolysis, mannitol, as a free radical scavenger and membrane stabilizer, was added to SAG, resulting in the synthesis of SAGM (Saline, Adenine, Glucose, Mannitol) solution [114]. Eventually, other well-known solutions, i.e., AS-1 and AS-5, were synthesized from SAGM by changing the concentrations of salt, sugar, and mannitol [110]. AS-3 is another additive solution that is prepared by adding citrate and phosphate to SAG. Indeed, citrate in AS-3 plays the same role as mannitol in SAGM; however, citrate can also balance the osmotic pressure of cells. In addition, AS-3 uses citrate phosphate double dextrose (CP2D) as an anticoagulant, which has a higher dextrose content than CPD. It is noteworthy that the membrane proteins of RBCs appear to be preserved better in AS-3 than in SAGM [115].

4.2. RSL tests for stored RBCCs

Among the most important RSLs, we can mention glucose consumption, lactate accumulation, reduced pH, hemolysis-induced ROSs, protein and carbohydrate oxidation, lipid peroxidation, release of microvesicles, potassium leakage, increased intracellular calcium, and reduction of ATP and 2,3-diphosphoglycerate (2,3-DPG) [45, 116]. These unwanted events can affect the oxygen delivery function of RBCs by the Bohr effect, or reduce in-vivo viability of cells [116, 117]. Thus, it is important to measure the survival and function of stored RBCCs before and after transfusion. In the following, some of the most important tests to measure the quality of these concentrates have been introduced.

4.2.1. Viability tests

The percentage of hemolysis is one of the most important factors in evaluating RBC survival. Hemolysis can be induced by storage time, filtration procedure, bacterial contamination, and increased penicillin and vitamin C levels in donated blood [118]. Leukocytes can lyse RBCs during storage by increasing the release of hydrogen peroxide and protease; thus, non-leukoreduced concentrates may show higher hemolysis than leukoreduced units [119]. Hemolysis prevents the release of oxygen in the tissues by increasing the oxygen affinity of hemoglobin. Moreover, biochemical reactions between free hemoglobin (Hb) and nitric oxide lead to endothelial dysfunction, thrombosis, and vasoconstriction [118].

4.2.2. Biochemical tests

Based on the Bohr effect, 2,3-DPG might affect the quality of RBCCs by altering the oxyhemoglobin dissociation curve [117]. Indeed, the acidosis induced by glycolysis can gradually decrease the pH value of RBCCs in the middle of storage time. This acidosis can shift the curve to the right, resulting in a decrease in 2,3-DPG. Hence, the reduction in 2,3-DPG can shift the curve to the left and increase the affinity of oxygen for hemoglobin. Interestingly, the normal level of 2,3-DPG recovered 1 week after blood transfusion. However, glycolysis is gradually slowed down by the accumulation of lactate and H^+, leading to a reduction in ATP synthesis. After 5 weeks, the ATP level can be reduced by 60% in stored RBCCs [45]. The reduced ATP level can then shift the biconcave discocyte RBC to echinocytes and release microvesicles. By adjusting the pH or

warming up the concentrates, this effect can be reversed [120]. Therefore, it should be clear that maintaining the pH of stored RBCCs in the range of 6.4–7.4 is a necessity, which may be achieved using additive solutions. Decreased ATP levels not only change the morphology of blood cells but also affect pathways that require ATP, such as membrane phospholipid distribution, membrane pumps, and antioxidant reactions [45, 121]. Disruption of the energy production process leads to an increase in intracellular Ca^{2+} because ATP is necessary for the activity of Ca^{+2} pumps. In addition to Ca^{+2}, long-term storage of RBC disrupts cation homeostasis by increasing the leakage of K^+ and reducing extracellular Na^+ [121]. Indeed, the hypothermic storage of RBCCs makes it possible to reduce cell metabolism and therefore increase the storage period up to 42 days. Thus, the activity of the Na^+/K^+ pump can be decreased, which in turn results in an increase in extracellular K^+ and the entry of Na^+ into the cells. Although the activity of these enzymes is recovered after transfusion, hyperkalemia can be problematic in some recipients, including children [45]. In addition, as the storage time of blood cells increases, byproducts such as ROS increase in the cells. During the first two weeks of storage, the metabolites of the pentose phosphate pathway (PPP) are increased in order to strengthen the body's antioxidant defense by producing NADPH. However, after two weeks, RBCs lose this ability and ROS levels increase more and more in the stored cells. As oxidative stress increases, RBC proteins, lipids, and carbohydrates can be oxidized [121]. Band 3 is a protein that can cooperate with carbonic anhydrase and hemoglobin to maintain the oxygen delivery function of RBCs. For this purpose, carbonic anhydrase converts CO2 into bicarbonate, which in turn can be hydrolyzed into H^+ and bicarbonate. In the following, band 3 exchanges intracellular bicarbonate with plasma Cl^-, resulting in transient intracellular acidosis. This decrease in intracellular pH leads to the release of oxygen from hemoglobin [122, 123]. Although the activity of band 3 is decreased in stored RBCCs between 14 and 35 days, it can be restored after transfusion [124]. Glycosylation and oxidation of membrane proteins also induce some rheological changes in RBCs. The reason is that spectrin can be oxidized and β-chain of hemoglobin can be covalently attached to the cytosolic tail of band 3, which in turn weakens the cytoskeleton. In addition to proteins, oxidative stress can target lipids [116]. Using metabolomics, it has been demonstrated that the metabolic regulation of amino acid precursors of glutathione, i.e., glutamate and cysteine, is disrupted with an increase in the storage time [121].

4.2.3. Morphological tests

Long-term storage of RBCCs leads to changes in the protein and lipid composition of the membrane, which facilitates vesiculation. The release of microvesicles after the second week leads to a change in the shape of RBCs from biconcave discocytes to echinocytes. This outcome reduces the deformability of RBCs as well as their response to osmotic stress. After transfusion, this reduced deformability can prevent RBCs from being passed through blood capillaries. Therefore, these old RBCs cannot be cleared by the spleen [116]. Alternatively, these cells may undergo osmotic stress-induced apoptosis. Moreover, the presence of phosphatidylserine and CD47 on the surface of these cells makes them susceptible to be phagocytized by the spleen and liver [121]. Ektacytometry is an approach that can be used to study RBC deformability [125]. Counting the microparticles released from the RBC during the storage period is another quality test. Prothrombotic and proinflammatory microparticles, which are released by apoptotic RBCs, have phosphatidylserine on the outer surface of their membrane. It is noteworthy that normal RBCs can also release some vesicles rich in oxidized lipids in the middle of their lifespan [116].

4.2.4. In-vivo efficacy tests

The standard method for determining the viability of RBCCs is the ^{51}Cr-radiolabeled RBC 24-h in-vivo recovery [116].

4.3. Quality testing of irradiated RBCCs under various storage conditions

Various storage conditions and timings of radiation can induce different RSLs in RBCCs, which are briefly explained below (details are shown in Tables 2 and 3).

4.3.1. Post-storage irradiated RBCCs

To ensure the quality of the RBCC units, the process of irradiation is performed precisely one day after their collection. Subsequently, to conduct thorough testing, samples are extracted from the aforementioned units on four separate occasions. These occasions consisted of day 0 (prior to radiation), serving as the control group, 1, 14, and 28 days after radiation. It is important to note that day 0 is specifically defined as 1 day after the collection of whole blood when screening test results are determined.

Patidar et al. have examined the quality of irradiated PRBCs stored either in CPDA-1 or SAMG by analyzing their biochemical parameters over a storage period of up to 28 days after irradiation. Irradiating both the CPDA-1-preserved and SAGM-preserved RBCs on day 1 of collection and then storing them for 28 days increased extracellular K^+ and reduced extracellular Na^+ from day 1 of storage. After 7 days, an increase in K^+ level was significant between the irradiated and non-irradiated units. Although an increase in lactate was significant between the irradiated and non-irradiated CPDA-1-preserved RBCs after 14 days, it was significant between the irradiated and non-irradiated SAGM-preserved RBCs from day 1 [126]. While SAGM-preserved RBCs showed higher mean ATP than CPDA-1-preserved RBCs, the irradiated CPDA-1-preserved RBCs showed higher pH values than the irradiated SAGM-preserved RBCs on day 7 of storage. Interestingly, the glucose level was closely doubled in the irradiated SAGM-preserved PRBCs compared with the irradiated CPDA-1-preserved PRBCs, which was attributed to the different amounts of dextrose in these solutions. Moreover, irradiated CPDA-1-preserved RBCs showed higher free-Hb than SAGM-preserved RBCs [126]. In another study aimed to assess the impact of irradiation on the quantity of MPs present within transfusion components, it has been shown that the number of CD41+ MPs increased in CPDA-1-preserved RBCs, whereas the number of CD235a+ MPs decreased. Indeed, CD41+ MPs appeared to have procoagulant and inflammatory activities [127]. Irradiating either the CPDA-1-preserved RBCs or the SAGM-preserved RBCs on day 7 of collection and then storing them for 35 days could also increase K^+ leakage and reduce extracellular Na^+ from day 1 of storage in both irradiated and non-irradiated units. There was also an increase in lactate levels from day 1 of storage in all units, but this increase was significant between the irradiated and non-irradiated units from day 14, regardless of the additive solutions. While the glucose level was significantly reduced in the irradiated CPDA-1-preserved RBCs after 28 days, it dramatically declined in the irradiated SAGM-preserved RBCs after 14 days. In addition, the pH value was significantly reduced in the irradiated CPDA-1-preserved RBCs on days 14 and 35, whereas this reduction in pH was observed in the irradiated SAGM-preserved RBCs from day 7. Interestingly, although there was no significant decrease in ATP levels between the irradiated and non-irradiated CPDA-1-preserved RBCs, ATP levels were significantly reduced in the irradiated SAGM-preserved RBCs compared with the non-irradiated units from day 14. Under this storage condition, free-Hb was significantly increased in irradiated CPDA-1-preserved RBCs from day 14, but it was significantly reduced in irradiated SAGM-preserved RBCs from day 7. They concluded that SAGM-PRBCs exhibited superior stability following irradiation in comparison to

CPDA-1 PRBCs. The safety thresholds for CPDA-1 PRBCs seemed to extend up to a period of two weeks after irradiation. Conversely, SAGM-PRBCs demonstrated acceptable safety thresholds for a duration of up to three weeks following irradiation [126].

Eshghifar et al. investigated the effects of irradiation on RBCs at different storage durations following blood collection. They demonstrated that irradiating CPDA-1-preserved RBCs on day 2 of collection and then storing them until days 2, 9, and 16 resulted in higher K+ leakage in the irradiated units than in the non-irradiated ones. Moreover, the level of extracellular Na+ was lower in the irradiated units on days 9 and 16. The level of 2,3-DPG was reduced in both the irradiated and non-irradiated units, but it was higher in the irradiated units on day 16. However, the levels of LDH and hemolysis in the irradiated unit were similar to those in the non-irradiated units. Irradiating CPDA-1-preserved RBCs on day 9 of collection and then storing them until days 9, 16, and 23 showed higher K+ leakage and lower extracellular Na+ in the irradiated units than in the non-irradiated ones. The level of 2,3-DPG declined in both irradiated and non-irradiated units, but this reduction was higher in all irradiated units than in non-irradiated units. There was no significant difference in LDH levels and hemolysis between the irradiated and non-irradiated units. Irradiating CPDA-1-preserved RBCs on day 14 of collection and then storing them until days 14, 21, and 28 yielded the same results. Based on their investigation, they suggested that a storage duration of up to 28 days after irradiation is permissible. Furthermore, the optimal timing for irradiation following blood collection was reported to be within 14 days. It is important to note that the blood unit should be transfused as soon as possible after irradiation [128].

Moreover, in a study conducted by Cicha et al. RBCs in mannitol-adenine-phosphate (MAP) medium were subjected to 35 Gy of irradiation and stored at 4 degrees C for a duration of 4 weeks. Irradiation of MAP-preserved RBC units on day 1 of collection reduced extracellular Na^+ and increased K^+ leakage; however, these changes were higher for irradiated units than for non-irradiated ones from day 8. No hemolysis was observed in any unit for up to 4 weeks. The number of echinocytes increased in both irradiated and non-irradiated units. Moreover, storage slightly increased lipid peroxidation and membrane protein aggregation in both irradiated and non-irradiated units. The spectrin and actin fractions were reduced by storing blood cells, whereas the band 3, band 6, protein 4.1, and protein 4.2 fractions were increased in both the irradiated and non-irradiated units. They concluded that the reduced deformability of gamma-ray-irradiated RBCs in MAP medium was primarily caused by dehydration due to potassium loss. Moreover, the membrane lipids and proteins of the RBCs remained stable and were not significantly affected by oxidative stress [129]. In addition, irradiating AS-1-preserved RBCs either on day 1 or on day 28 of collection could increase K^+ leakage [130].

El Kenz et al. assessed the effects of gamma irradiation on PRBCs stored in AS-3 medium, which is commonly used for neonatal transfusion due to its perceived safety. Irradiation of RBCCs using a ^{60}Co-irradiator showed that CPDA-1-preserved RBCs had higher K^+ leakage and lower extracellular Na^+ than SAGM-preserved RBCs in both irradiated and non-irradiated units; however, there was no difference between the irradiated and non-irradiated units. The irradiated CPDA-1-preserved RBCs revealed lower glucose levels than the irradiated SAGM-preserved RBCs; however, there was no significant difference in glucose levels between the non-irradiated CPDA-1-preserved RBCs and the non-irradiated SAGM-preserved RBCs. The irradiated CPDA-1-preserved RBCs had higher lactate and LDH levels than the irradiated SAGM-preserved RBCs. The non-irradiated CPDA-1-preserved RBCs had higher lactate levels than the non-irradiated SAGM-preserved RBCs. On day 28, LDH levels were slightly higher in irradiated SAGM-preserved RBCs, but there was no significant difference in LDH levels between non-irradiated

CPDA-1-preserved RBCs and non-irradiated SAGM-preserved RBCs. They concluded that caution should be exercised when considering the use of irradiated PRBCs stored in AS-3 beyond 7 days post-irradiation, particularly in the context of massive and/or rapidly infused transfusions in neonates and infants [131].

4.3.2. Leucoreduced post-storage irradiated RBCCs

In a study aimed to assess the effects of early irradiation (day +3) on leucoreduced red blood cell units and the damages incurred during storage, it was shown that irradiating SAGM-preserved RBCs on day 3 of collection could increase K^+ leakage in irradiated units more than in non-irradiated units from day 7 of storage [132]. In addition, two other similar studies demonstrated that irradiating SAGM-preserved RBCs on days 14, 28, and 35 of collection could also increase K^+ level upon irradiation. These units exhibited higher LDH levels and hemolysis than non-irradiated units [133, 134]. Irradiating SAGM-preserved RBCs between days 8 and 40 of collection and then storing them for 28 days enhanced K^+ leakage. The highest level of hemolysis was observed in the units irradiated in the 4th week and then stored for 15–21 days. In total, the recently irradiated units showed higher hemolysis than the early irradiated units [135, 136]. Irradiating phosphate-adenine-glucose-guanosin-saline-mannitol (PAGGS-M)-preserved RBCs on day 14 of collection and then sampling within 28 days could improve K^+ leakage. The levels of glucose and lactate in these irradiated units were similar to those in the non-irradiated units until day 49. ATP was increased in both irradiated and non-irradiated units until day 14 and then started to reduce gradually from day 21. Moreover, 2,3-DPG was reduced in both irradiated and non-irradiated units until day 14. From day 21 of storage, a higher hemolysis was observed in the irradiated units; however, it was comparable between both the irradiated and non-irradiated units on day 49. After day 35, the LDH level was higher in the irradiated units than in the non-irradiated units [136]. It can be concluded that early irradiation does not result in more damage to RBCs during subsequent storage compared to irradiation on day +14. Nevertheless, the maximum storage period for irradiated RBCs should be limited to 28 days from the date of collection, regardless of the day of irradiation within this period [136].

4.3.3. Pre-storage irradiated RBCCs

Irradiating the SAGM-preserved RBCs collected by the buffy coat method and then storing them for up to 42 days increased cytosolic Ca^{2+} in these units more than the non-irradiated ones. From day 21 to day 42 of storage, the ATP level was reduced more in the irradiated units. Free-Hb, mean corpuscular volume (MCV), and microvesicle release were also higher in the irradiated units than in the non-irradiated units during this storage period. Under energy depletion conditions, 4- to 21-day-old irradiated units showed more phosphatidylserine (PS) externalization than non-irradiated units [137]. Maia et al. investigated the involvement of Na,K-ATPase in potassium leakage from prophylactically irradiated RBCs. They irradiated RBCCs with 25Gy of γ-radiation within 24 hours of collection. At various time points post-irradiation (days 3, 5, 7, 9, 11, 14, and 28), fractions were removed and centrifuged to determine Na,K-ATPase activity from ghost membranes. Irradiation and storage of CPDA-1-preserved RBCCs revealed that Na^+/K^+-ATPase activity was reduced by 12.6% and 50% on days 7 and 14 of storage, respectively [138]. In addition, Barjas-castro et al. used laser optical tweezers to investigate the elastic properties of irradiated and stored RBC units. It has been shown that there was no difference in membrane elasticity between the irradiated and non-irradiated units until day 14, but it could be reduced more in the irradiated cells after day 21 [139].

Irradiation of CPDA-1- or SAGM-preserved RBC units using a ^{60}Co irradiator reduced the surface roughness of RBCs, and these cells became more rigid in response to the irradiation dose and storage time. Morphological tests of the irradiated RBCs revealed small nanopores, protrusions, and potholes. On day 5 of storage, the numbers of echinocytes and ovalocytes increased [140, 141]. In addition, two similar studies investigated the impact of gamma irradiation on the hematological and biochemical parameters of allogeneic red blood cell units during storage. Results revealed that on days 1, 7, 14, and 21 of storage, lipid peroxidation in the irradiated units was similar to that in the non-irradiated units. Although protein carbonylation could not be seen on day 1 of storage, it was increased by 25% after 21 days in the irradiated units, which was more than that in the non-irradiated units [142, 143]. Moreover, protein thiol levels in the membrane were dose-dependently increased in the irradiated units from day 1 of storage [131, 142, 143]. In another study, the irradiated AS-3-preserved RBC units could remain at the allowable K^+ content, i.e., 7.0 mM, for up to 14 days. Moreover, the level of free-Hb in these units remained within the allowable range up to day 21 [131].

4.3.4. Leucoreduced, pre-storage irradiated RBCCs

Studies conducted to evaluate the effects of irradiation on the alterations associated with the RBC storage lesions showed that storing RBCCs from day 1 to day 42 of irradiation enhanced K^+ leakage in WB, RBCCs, and leucocyte-reduced RBCCs compared with their non-irradiated counterparts; however, this effect was not significant for 28-day-old WB. All irradiated units exhibited lower extracellular Na^+ levels than their non-irradiated counterparts. The pH value decreased in both leucoreduced irradiated and non-irradiated units, but this reduction was higher in leucoreduced irradiated units. 2,3-DPG was reduced rapidly until day 21 for both leucoreduced irradiated and non-irradiated units. Higher LDH was observed in the 14-, 28-, and 42-day-old leucoreduced irradiated units compared with the non-irradiated units. In contrast to the non-irradiated units, all irradiated concentrates showed more than 0.8% hemolysis on days 35 and 42 of storage [118, 144]. From day 3 to day 28 of storage, there was no cross-linking/fragmentation of hemoglobin in leucoreduced, irradiated RBCCs, but there was ferrous center oxidation, which was significantly increased between days 5 and 7 of storage. After day 9, this ferrous center oxidation was reduced and reached that of in the non-irradiated [40]. Pre-storage irradiated paediatric RBCCs also showed higher K^+ leakage and lower extracellular Na^+ compared with their non-irradiated units; however, this increase in K^+ leakage was not significant for 42-day-old paediatric RBCCs. The 42-day-old paediatric RBCCs had a higher LDH level and hemolysis than their non-irradiated counterparts [118]. In addition, Leitner et al. examined the impact of gamma irradiation on the intracellular purine nucleotides of red blood cells during storage. They have shown that storing leucoreduced irradiated SAGM-preserved RBCCs could reduce purine nucleotide metabolism in RBCs. ATP, ADP, and GTP levels increased during the first 7 days of storage and then gradually began to decrease until 39 days. The reduction in the ATP and ADP levels of the irradiated units was more significant than that of the non-irradiated units. ATP was also increased in both the irradiated and non-irradiated units until day 7 and then decreased until day 39. Hemolysis was higher in the irradiated units than in the non-irradiated units. The free-Hb level was higher in the leucoreduced, irradiated units until day 21, and then it started to reduce [145]. In a metabolomic analysis comparing the patterns of metabolites between gamma-irradiated and nonirradiated CPDA-1-split RBCs a storage period of 35 days, there was no significant difference between leucoreduced irradiated CPDA-1-preserved RBCs and non-irradiated RBCs until 7 days of storage. However, after day 10, the metabolomics of the irradiated units were similar

to those of the older non-irradiated units [146]. In addition, proteomic analysis of RBC concentrates subjected to 30 Gy of irradiation and stored at a temperature of 4 ± 2°C for either 1 or 15 days was conducted. Results demonstrated that irradiating and then storing leucoreduced PAGGS-M-preserved RBCs showed that there was no significant difference in proteomics data between the irradiated and non-irradiated units on day 1 of storage; however, 27 protein spots were altered on day 15 of storage. The protein abundances of DDB1, VCP, and TGase 2 were reduced in both the irradiated and non-irradiated units [147]. From day 1 to day 35 of storage, the numbers of pseudopodia and debris increased in leucoreduced irradiated units compared with non-leucoreduced non-irradiated units. After 42 days of storage, leucoreduced AS-preserved RBCs were irradiated. Based on the data, the extracellular K^+ level was higher in the non-leucoreduced, irradiated units than in the non-irradiated, leucoreduced units on day 42 of storage. Moreover, the leucoreduced, irradiated AS-3- and AS-1-preserved RBCs showed less glucose reduction. The non-leucoreduced, irradiated units showed lower ATP levels than the non-irradiated, leucoreduced units. In addition, the leucoreduced, irradiated AS-3-preserved RBCs showed less ATP reduction. The non-leucoreduced, irradiated units exhibited higher hemolysis than the non-irradiated, leucoreduced units. Leucoreduced irradiated AS-1/AS-3-preserved RBCs exhibited less hemolysis [148].

4.3.5. Washing irradiated RBCs Vs. irradiating washed RBCs

Some researchers [149] have evaluated the effects of "washing after irradiation" or "washing before irradiation" protocols on the quality of stored RBCCs one day after their expiry date. They showed that the level of extracellular K^+ was improved in both approaches within 24 h, but it was increased less in "washing after irradiation" protocol. K^+ levels can reach above 5 mEq/L in a time-dependent manner after washing the irradiated units. During a 6-h storage period after washing, there is a very low possibility for washed non-irradiated units to show a K^+ level greater than 5 mEq/L. This time for irradiated and washed RBCCs is approximately 3 h (Table 4).

5. CONCLUSIONS

Storage conditions and age of blood products at the time of gamma irradiation are key factors that can affect the quality of transfused blood. Until day 5, pre-storage gamma irradiation of apheresis- and PRP-obtained PCs using both ^{60}Co- and ^{137}Cs-irradiators did not induce any changes in in-vitro quality of PCs compared with non-irradiated units. The post-storage of apheresis PCs within 5 days of irradiation also indicated no significant difference in in-vitro quality of PCs compared with non-irradiated units. In comparison with irradiated PCs, leukoreduced PCT PCs showed lower cell counts and glucose levels, as well as higher LDH and lactate levels, especially after day 7 of storage. Moreover, platelet activation and degradation appear to start earlier in PCT PCs during storage. In comparison with irradiated PCs, PRT PCs exhibited higher glycolytic and oxidative respiration activities. However, compared with untreated PCs, both irradiated and PRT PCs accelerated storage lesions at the protein/metabolic level. The post-storage irradiation of RBCCs using irradiators that use either ^{137}Cs or ^{60}Co has shown that the irradiated SAGM-preserved RBCCs have better stability than the CPDA-1-preserved RBCCs. Moreover, other mannitol-containing additive solutions, e.g., MAP, indicate less membrane lipid peroxidation, which is attributed to the ROS scavenging activity of mannitol. Thus, it is important to determine the shelf life of irradiated RBCCs according to the additive solution used. Moreover, the age of irradiated RBCs and the day of irradiation after both collection and leukoreduction can also affect the quality of RBBCs. It is noteworthy to mention, RBBCs irradiated on day +14 can induce too

many RSLs, regardless of the additive solution. After irradiation, leucoreduced RBCCs showed better quality, indicating the inhibition of hemolysis mediated by leukocytes in RBCs. In addition, washing can affect the quality of RBCCs in a time-dependent manner, which is higher after irradiating washed RBCCs than vice versa. Finally, it is of utmost importance to consider timing of irradiation and storage conditions, such as preparation method, anticoagulants/additive solution, temperature, type of blood bags, filtration, and washing, in the production of irradiated PCs and RBCCs.

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Table 1: Effects of pre-storage (day 0) gamma irradiation on platelet yield, activity, function, morphology, and cellular organic and inorganic molecules (follow up from day 1 to 7)

No.	Study design	~~Results~~	platelet activation and function	Platelet morphology	Other findings	Ref.
1	Pre-storage irradiated apheresis PLTs vs. non-irradiated ones	✓ Platelet yield: ↓ slightly on the 5th day ✓ MPV: ↓ on Day 1; fairly constant until 7 days ✓ pH: 6.4-7.4	Day 1-5: -Ca2+: no difference** -CD62P: ↑ expression*	swirling scores: no difference**	Day 1-5: pO2, pCO2, Na+, K+, and HCO3: no difference** -glucose: ↓ ** on day 5 -lactate: ↑ ** on day 5 Day 5-7: -lactate: higher* -glucose: lower* -LDH: higher* Day 7: -pCO2: lower*	[91]
2		-	Day 1-5: HSR: ↓ over time** Day 1-7: GPIb: well-maintained** -P-selectin: ↑ expression**, especially irradiated ones	-	-	[92]
3	Pre-storage irradiated vs. non-irradiated plasma-rich PLTs	Day 1-3: ✓ PLT yield: no difference**	Day 1: ✓ CD62p: ↑ expression* Day 3: ✓ CD62p: ↑ expression*	-	-	[94]
4		Platelet yield, MPV, PDW and viability: no difference**	Day 2: ✓ HSR: normal ✓ α angle: normal ✓ Higher R and K values with prolongation of storage time; ** ✓ ADP/collagen-induced aggregation: ↓, but there was no difference **	Day 2: ✓ Morphological score: almost normal, with ballooned and capping shapes	✓ pH: dropped, but there was no difference ** ✓ pCO2: higher *	[95]
5	Pre-storage irradiated apheresis PLTs vs. photochemically treated apheresis PLTs	Day 1-7: PLT yield: ↓ ***, but not in irradiated units pH: 6.4-7.4**	✓ Day 1-7: ✓ CD61+ microparticles and CD62P+ PLT: higher*** ✓ CD42+ PLT: lower *** than irradiated units ✓ ADP/TRAP-induced CD62P expression: ↓ *** ✓ ADP/TRAP-induced CD42b downregulation: ↓ *** ✓ TRAP-induced CD61+ microparticle formation: slightly higher *** after 5 and 7 days ✓ Aggregation and velocity in response to collagen (25 mg/Ml): lower ***	✓ Swirling scores: ↓ **	Day 1-17: ✓ LDH: ↑ ***, but not in irradiated units ✓ Low glucose and high lactate *** after 7 days ✓ Lower pCO2 and HCO3, as well as higher pO2 *** until 7 days ✓ PLT-derived cytokines: high **, but higher *** ✓ WBC-associated cytokine: low or undetectable **	[51]
6		Day 1-7: ✓ Platelet yield: reduced ***, but not in irradiated units	Day 1-7: ✓ CD61+ microparticles and CD62P+ PLT: higher ***	-	Day 1-17: ✓ LDH: ↑ ***, but not in irradiated units ✓ PLT-derived cytokines: high ** groups, but higher *** ✓ WBC-associated cytokine: low or undetectable **	[99]
7	Pre-storage irradiated vs. Mirasol riboflavin-based pathogen reduction technology (PRT) treated apheresis PCs	✓ PLT count: no difference among unites	✓ Ristocetin-induced aggregation: ↓ in all units ✓ Collagen-induced P-selectin: ↑ in all units, especially // ✓ Lower mitochondria membrane polarization //, but not after irradiation	Day 5: ✓ Lower swirling scores for // than irradiated and non-irradiated units	✓ K+ and LDH: no difference among unites ✓ Lower glucose and higher lactate // ✓ Lower pO2 and higher pCO2 in PRT PCs until 5 days, but all units maintained pO2 above 10 mm Hg	[101]
8		-	-	-	✓ Storage, irradiation, and PRT: change proteome and metabolome	[105, 107]

*: in irradiated units compared to non-irradiated unites, **: in both groups, ***: in PCT PCs, //: in PRT PCs, PLT: platelet, PC: platelet concentrate, PCT PCs: photochemically treated platelet concentrates

Table 2: Effects of pre-storage (day 0) gamma irradiation using Cesium-137 as a gamma source on RBC ion levels, ATP synthesis, hemolysis and 2,3-DPG, and morphology (follow up from day 1 to 28)

No.	Processing conditions	Na/K/Ca levels	ATP synthesis	Hemolysis and 2,3-DPG	Morphology	Ref.
1	leucocytereduced RBCCs	Day 1-28: ✓ K+ leakage: higher in WB, RBCC, and leucocytereduced RBCC than non-irradiated units ✓ Extracellular Na+: lower *	Day 1-28: ✓ Low glucose ** ✓ Low pH **	Day 1-28: ✓ High LDH ** ✓ Higher LDH in 14- and 28-day-old leucoreduced *		[118, 144]
2					Day 3-28: ✓ No cross-linking and fragmentation in hemoglobin ✓ Presence of a ferrous center oxidation, especially between 5 and 7 days of storage	[150]
3	SAGM-preserved RBCs collected by buffy coat method		Day 21-28: ATP level: lower *	Day 21-28: Free-Hb: higher *	Day 21-28: MCV and microvesicle release: higher *	[137]
4	Paediatric RBCCs	Day 1-28: ✓ K+ leakage: higher in paediatric RBCCs than non-irradiated units ✓ Extracellular Na+: lower *	Day 1-28: ✓ Low glucose **	Day 1-28: High LDH **		[118]
5	Leucoreduced SAGM-preserved RBCs	Day 1: ✓ K+ leakage: higher ✓ Extracellular Na+: ↓ **	✓ high ATP ** 7 days ✓ Reduction in ATP level: more * from day 21	✓ Hemolysis: higher * ✓ Free-Hb: higher in leucoreduced * units until day 21		[145]
6	CPDA-1-preserved RBCs	Day 7 and 14: ✓ Na+,K+-ATPase activity: ↓ Day 21 and 28: ✓ A slight ↑ in inhibition of the pump				[138]
7					✓ Membrane elasticity: Similar ** until day 14 ✓ Membrane elasticity: ↓ more * units after day 21	[139]
8	Leucoreduced CPDA-1-preserved RBCs:		✓ Metabolomic profiles: no difference ** until 7 days			[146]
9	Leucoreduced PAGGS-M preserved RBCs:				Day 1: ✓ Proteomics: no difference ** ✓ Alteration in 27 protein spots on Day 15 of storage ✓ DDB1, VCP, and TGase 2 protein abundances: ↓ after irradiation and storage Day 1-28: ✓ Pseudopodia and debris: ↑ in both irradiated and filtrated units	[147]

*: in irradiated unites compared to non-irradiated unites, **: in both irradiated and non-irradiated unites

Table 3: Effects of pre-storage (day 0) gamma irradiation using Cobalt-137 as a gamma source on RBC ion levels, ATP synthesis, hemolysis and 2,3-DPG, and morphology (follow up from day 1 to 28)

No.	Processing conditions	Na/K/Ca levels	ATP synthesis	Hemolysis and 2,3-DPG	Morphology	Other findings	Ref.
1	CPDA-1- or SAGM-preserved RBC units	Day 1-14: ✓ Extracellular Na+: lower * ✓ K+ leakage: higher * >14 Days: ✓ Less Na+ and higher K+ ** compared to those of <14 Day of storage		Day 1-14: ✓ Free-Hb and LDH: higher * >14 Days: ✓ More LDH and free-Hb ** compared to those of <14 Day of storage		Days 1, 7, 14, and 21: ✓ Lipid peroxidation: no difference ** ✓ No protein carbonylation on day 1 of irradiation ✓ Higher protein thiol levels in membrane of irradiated RBCs from day 1 of storage	[142, 143]
2					Day 5: More echinocytes and ovalocytes		[140, 141]
3	AS-3-preserved RBC units	✓ K+ level: remained at allowable content up to day 14	-	✓ Free-Hb: remained at allowable range up to day 21	-		[131]

*: in irradiated unites compared to non-irradiated unites, **: in both irradiated and non-irradiated unites

Table 4: Detailed information regarding gamma-irradiation in reviewed studies, including model of irradiator, gamma ray source, dose, and time of exposure

No.	Model of irradiator	Gamma ray source	Dose	Time of irradiation	Ref.
1	Gammacell 3000 Elan, Nordion International Inc., Ottawa, Ontario, Canada	Cs-137	25 Gy	-	[51]
2	Gammacell 3000 Elan, Nordion, Canada	Cs-137	25 Gy	-	[91]
3	Gammacell 3000 Elan, MDS Nordion, Ottawa, Ontario, Canada	Cs-137	25 Gy	-	[92]
4	-	Cs-137	25 Gy	-	[94]
5	-	Co-60	25 Gy	-	[95]
6	Gammacell 3000 Elan, Nordion International Inc., Ottawa, Ontario, Canada	Cs-137	25 Gy	-	[99]
7	-	Cs-137	30 Gy	-	[101]
8	-	Cs-137	30 Gy	-	[107]
9	Gammacell 3000 irradiator (Nordion Inc., Ottawa, Ontario, Canada)	Cs-137	✓ Minimal: 2574 cGy ✓ Central dose: 2932 cGy	-	[118]
10	-	Co-60	25 Gy	-	[131]
11	-	Cs-137	25 Gy	-	[137]
12	-	Cs-137	25Gy	-	[138]
13	IBL 437C irradiator, Cis Bio International, Gif sur Yvette, France	Cs-137	25 Gy	-	[139]
14	-	Co-60	10, 15, 20, 25, and 30 Gy	-	[140]
15	Nordion, Gammacell 220 Excel, Canada	Co-60	15, 25, 35, and 50 Gy.	-	[141]
16	-	Co-60	25 Gy	-	[142]
17	first category Irradiator BK-10000 ZZUJ Polon, Poznan, Poland	Co-60	30, 40, and 50 Gy	-	[143]
18	IBL 437 CSI Biointernational®; Cedex, Paris	Cs-137	30 Gy	-	[145]
19	Nordion Gamma irradiator (Nordion, Ottawa, ON, Canada)	Cs-137	25 Gy	-	[146]
20	-	Cs-137	30 Gy	20 min	[147]
21	Mark 1, J.L. Shepherd & Associates, San Fernando, CA, USA	Cs-137	25 Gy	-	[148]
22	-	Cs-137	25 Gy	-	[150]