Transfusion-related immunomodulation in patients with cancer: Focus on the impact of extracellular vesicles from stored red blood cells (Review)

XINGYU MA[1], YANXI LIU[1], QIANLAN HAN[1], YUNWEI HAN[2], JING WANG[3] and HONGWEI ZHANG[3]

[1]Class 2018 Medical Inspection Technology, Southwest Medical University; Departments of [2]Oncology and [3]Blood Transfusion, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

Received August 4, 2021; Accepted November 5, 2021

DOI: 10.3892/ijo.2021.5288

Abstract. Red blood cell (RBC) transfusions may have a negative impact on the prognosis of patients with cancer, where transfusion-related immunomodulation (TRIM) may be a significant contributing factor. A number of components have been indicated to be associated with TRIM. Among these, the impact of extracellular vesicles (EVs) has been garnering increasing attention from researchers. EVs are defined as nano-scale, cell-derived vesicles that carry a variety of bioactive molecules, including proteins, nucleic acids and lipids, to mediate cell-to-cell communication and exert immunoregulatory functions. RBCs in storage constitutively secrete EVs, which serve an important role in TRIM in patients with cancer receiving a blood transfusion. Therefore, the present review aimed to first summarize the available information on the biogenesis and characterization of EVs. Subsequently, the possible mechanisms of TRIM in patients with cancer and the impact of EVs on TRIM were discussed, aiming to provide an outlook for future studies, specifically for formulating recommendations for managing patients with cancer receiving RBC transfusions.

Contents

1. Introduction
2. Impact of RBC transfusions on the prognosis of patients with cancer
3. TRIM in patients with cancer receiving RBC transfusions
4. Biogenesis and characterization of stored RBC-derived EVs
5. Mechanism of stored RBC-derived EVs in TRIM in patients with cancer receiving transfusions
6. Outlook

Correspondence to: Mrs. Jing Wang or Mr. Hongwei Zhang, Department of Blood Transfusion, The Affiliated Hospital of Southwest Medical University, 25 Taiping Street, Jiangyang, Luzhou, Sichuan 646000, P.R. China
E-mail: wangjing088@swmu.edu.cn
E-mail: zhanghongwei2020@swmu.edu.cn

Key words: stored red blood cells, transfusion-related immunomodulation, extracellular vesicles, patients with cancer

1. Introduction

In 1981, Gantt first proposed that tumor antigens are similar to histocompatibility antigens such as histocompatibility antigens class II, based on a number of shared characteristics (1,2). In addition, he proposed that transfusion-induced immunosuppression is not selective for histocompatibility antigens, which may affect the prognosis of patients with malignancies (1). Allogeneic red blood cell (RBC) transfusion is a form of therapy similar to cell transplantation and it may induce immunosuppression, tumor recurrence and post-operative infections in patients with cancer (3). Therefore, transfusions, particularly RBC transfusions for patients with cancer, warrant further scrutiny. Anemia is a common clinical condition among patients with cancer and has an incidence rate of 40-90% in Turkey, the US and Europe (4,5). RBC transfusion is one of the primary treatment options for the management of anemia (6). In the clinic, patients with hematological/oncological diseases use up ~34% of the total RBC supply (7,8). However, despite its proven ability to increase hemoglobin (Hb) and hematocrit levels, RBC transfusion is associated with poor prognoses for patients with cancer (7,9). Specifically, the rates of 30-day post-operative mortality, major complications and prolonged duration of hospital stay for recipients of intraoperative blood transfusions have all been reported to be significantly increased compared to those with no transfusion (10). This is proposed to be the result of the immunomodulatory and proinflammatory effects of allogeneic RBC transfusions, known as transfusion-related immunomodulation (TRIM) (11-14).

RBC extracts may be stored at the blood bank for ≤35 days using citrate phosphate dextrose adenine-1 as the preservative solution, or for 42 days using Adsola-1 as the preservative solution (15,16). During storage, RBCs suffer energy depletion, reductions in pH, alterations in cation homeostasis and oxidative stress, leading to changes in RBC morphology and

function (15-18). This in turn promotes the release of extracellular vesicles (EVs) into the storage medium, the occurrence of which is referred to as RBC storage lesions (15-18). EVs are spherical particles that are encased within a lipid bilayer with diameters of 30-1,200 nm, which may be secreted by cells into the extracellular milieu either physically or under pathological conditions (19-22). RBC-derived EVs, which may contain RNAs, immunoglobulins, complement proteins and exposed phosphatidylserine (PS), may bind to recipient cells to mediate intercellular communication (22-26). It is this mechanism that has been proposed to activate TRIM in patients with cancer to worsen prognosis (13,14,27).

2. Impact of RBC transfusions on the prognosis of patients with cancer

Several studies have reported that perioperative transfusions are associated with poor prognosis in a number of multiple solid malignant tumors (Table I), including, but not limited to, colorectal cancer (28-31), gastric cancer (32), pancreatic cancer (33), lung cancer (34), epithelial ovarian cancer (12), non-metastatic renal cell carcinoma (35), diffuse malignant peritoneal mesothelioma (36) and pseudomyxoma peritonei (36). The most convincing evidence for this association between perioperative blood transfusion and tumor recurrence provided for colorectal cancer (37). Of note, the rates of postoperative complications, distant metastasis, cancer recurrence and post-operative mortality were all indicated to be increased in patients receiving perioperative RBC transfusions (28-31). In a previous retrospective analysis of patients with colorectal cancer who recently underwent radical resection, even after most, if not all of the known clinicopathological predictors were comprehensively factored into consideration, the overall mortality rate was still significantly associated with perioperative transfusions, although it was not associated with preoperative anemia (38). In addition, Grasso *et al* (39) reported that intraoperative transfusions may increase the degree of immunomodulation due to surgical pressures for gastric cancer surgery, thus worsening prognosis and leading to the proposal that this procedure should be avoided if possible. This poor prognosis may also be dependent on the dose of RBC transfusions (34,35). A number of studies have previously indicated that patients with colorectal cancer who were transfused with ≥3 leukoreduced RBC units after surgery had lower overall survival and higher recurrence rates compared with those in patients who did not receive any transfusion or patients who received only 1 or 2 RBC transfusions (28,30,40,41). However, other factors, such as the cancer stage, rather than blood transfusions, are critical predictors of poor outcome following surgery for colorectal cancer (42-45).

The presence of leukocytes in RBC concentrates is one of the causes of adverse reactions post-transfusion (46). However, although the removal rate of leukocytes from leukoreduced RBC products may reach 99.9% (22), leukoreduction may only at best mitigate and not eliminate the negative impact of transfusions on patients with cancer (12,28). In patients with bladder cancer receiving neoadjuvant chemotherapy prior to radical cystectomy, perioperative transfusions with leukoreduced RBCs are associated with lower overall survival rates (47). The typical transfusion standard is a Hb concentration of ≤10 g/dl (48). However, several randomized trials have revealed that a conservative transfusion regimen (Hb concentration ≤7-8 g/dl) does not have any negative impact on the outcome of patients with cancer (49-52). In addition, another large oncology meta-analysis previously indicated that a restrictive transfusion policy may reduce the risk of perioperative transfusions by 36% without increasing the tumor recurrence or the mortality rate (53). Therefore, this restrictive threshold of transfusion should be clinically implemented to reduce the transfusion rates to maximize the impact on the survival of patients with cancer (12). A previous study indicated that transfusion with Hb ≥7 g/dl in hemodynamically stable patients is associated with increased risk of surgical site infection following rectal cancer surgery (54). However, a generous transfusion strategy (<9 g/dl) is recommended for patients with cancer coupled with infectious shock (55).

In conclusion, allogeneic RBC transfusions may have negative effects on the prognosis of patients with cancer and should be avoided. This is particularly the case during and following the operation, unless otherwise necessary. In terms of the impact of blood transfusions on the survival of patients with cancer, controversies remain and future studies should focus on the mechanism of TRIM, which is increasingly reported to be a major contributing factor of transfusion-related adverse events.

3. TRIM in patients with cancer receiving RBC transfusions

TRIM refers to a number of mediators that are able to interact with immune cells to alter their physiological function, including factors derived from residual leukocytes and platelets, hemolytic contents (heme and iron release) and EVs (14). To date, widespread observations of TRIM have been made in immunologically compromised groups of individuals, including patients with cancer, preterm neonates and critically ill children (28,56,57). TRIM causes symptoms by exerting immunosuppressive and proinflammatory effects (14). Prior to the availability of immunosuppressive drugs, allogeneic RBC transfusions were indicated to increase the survival rate of patients receiving kidney transplants (58,59). By contrast, in animal models, allogeneic blood transfusions were observed to significantly increase the size of the tumor (60), whilst reducing the removal rate of tumor cells (61). Previous clinical studies have reported that after patients with colorectal cancer or several other tumors received perioperative blood transfusions, the absolute number of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocyte subsets declined (30,62,63). For patients with gastric cancer who received perioperative allogeneic blood transfusions or autologous blood transfusions, plasma levels of neopterin, IFN-γ, T lymphocyte subsets ($CD3^+$, $CD4^+$) and the $CD4^+/CD8^+$ ratio were significantly decreased (63). However, patients who received allogeneic blood transfusions exhibited even lower levels compared with those who received autologous blood transfusions (63). In addition, in patients with nasopharyngeal carcinoma, a lower CD4/CD8 ratio was indicated to be associated with unfavorable prognosis (64).

Residual leukocytes, together with the immunoactive substances they release, have been reported to serve a role in TRIM (14,65). After blood transfusion, interaction between major histocompatibility complex II or human leukocyte antigen (HLA)-DR molecules and the recipient lymphocytes

Table I. Impact of transfusions on the survival of patients with cancer.

A, Transfusion-related immunomodulation

Author (year)	Title	(Refs.)
Al-Refaie *et al*, 2012	Blood transfusion and cancer surgery outcomes: A continued reason for concern.	(10)
Deeb *et al*, 2020	allogeneic leukocyte-reduced red blood cell transfusion is associated with postoperative infectious complications and cancer recurrence after colon cancer resection.	(28)
Tamini *et al*, 2021	Colon Cancer Surgery: Does preoperative blood transfusion influence short-term postoperative outcomes?	(29)
Qiu *et al*, 2016	Impact of perioperative blood transfusion on immune function and prognosis in colorectal cancer patients.	(30)
Acheson *et al*, 2012	Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: A systematic review and meta-analysis.	(31)
Liu *et al*, 2018	Effect of perioperative blood transfusion on prognosis of patients with gastric cancer: A retrospective analysis of a single center database.	(32)
Benson and Barnett, 2011	Perioperative blood transfusions promote pancreas cancer progression.	(33)
Churchhouse *et al*, 2012	Does blood transfusion increase the chance of recurrence in patients undergoing surgery for lung cancer?	(34)
Seon *et al*, 2020	Impact of perioperative blood transfusion on oncologic outcomes in patients with nonmetastatic renal cell carcinoma treated with curative nephrectomy: A retrospective analysis of a large, single-institutional cohort.	(35)
Nizri *et al*, 2018	Dose-dependent effect of red blood cells transfusion on perioperative and long-term outcomes in peritoneal surface malignancies treated with cytoreduction and HIPEC.	(36)
Cata *et al*, 2013	Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions.	(37)

B, No impact

Author (year)	Title	(Refs.)
Baguena *et al*, 2020	Impact of perioperative transfusions and sepsis on long-term oncologic outcomes after curative colon cancer resection. A retrospective analysis of a prospective database.	(42)
Tarantino *et al*, 2013	Blood transfusion does not adversely affect survival after elective colon cancer resection: A propensity score analysis.	(43)
Hunsicker *et al*, 2019	Transfusion of red blood cells does not impact progression-free and overall survival after surgery for ovarian cancer.	(44)
Zaw *et al*, 2017	Perioperative blood transfusion: Does it influence survival and cancer progression in metastatic spine tumor surgery?	(45)

A, Transfusion-related immunomodulation. Blood transfusions are associated with the poor prognosis in patients with cancer through blood transfusion-related immunomodulation, including colon cancer, gastric cancer and lung cancer. B, No impact. These studies have found that Transfusion of red blood cells does not impact progression and overall survival for patients with cancer, including colon cancer, ovarian cancer and metastatic spine tumor.

may lead to allogeneic immunity or immunomodulation (14). Dendritic cells expressing CD200 may stimulate recipient cells into secreting TGF-β (66), which is an immunosuppressive factor that has been associated with the escape of tumors from

immunosurveillance (67). Residual bioactive materials originating from $CD4^+$ T lymphocytes include immunomodulatory particles that contain large quantities of proinflammatory cytokines and chemokines, which may promote lymphoid hyperplasia and the generation of antibodies (68). In RBC products that have been stored for 30 days, large quantities of leukocyte-derived soluble HLA (sHLA)-I type antigens may be detected (69). sHLA-I molecules may in turn induce $CD8^+$ cell death to inhibit the cytotoxic activity of Epstein-Barr virus-specific $CD8^+$ cytotoxic T-lymphocytes (70) and neutrophil chemotaxis (71). Several proinflammatory molecules from RBC supernatants (72), including IL-1β, IL-6 and TNF-α, are able to promote the proliferation of HepG2 tumor cells (73) and the inflammatory cytokine response of peripheral blood mononuclear cells (74).

However, leukoreduction appears to be unable to eliminate TRIM (14). The survival rate of patients with epithelial ovarian cancer receiving pre-storage leukoreduced RBC units remains lower compared to that of such patients with no transfusion (12). A previous study indicated that leukoreduced RBC concentrates that have been previously stored inhibited the proliferation of $CD4^+CD8^+$ T cells and B cells *in vitro*, but their fresh pre-storage counterparts were able to reverse this suppression (75). In addition, pre-storage leukoreduced RBC supernatants have been documented to induce the activation of regulatory T cells, which may in turn inhibit the proliferation of T cells (72). Regulatory T cells have potent immunosuppressive activity to inhibit the anti-tumor immune response in the body (76).

Taken together, these data suggest that RBC transfusions may have a negative impact on the immunity of patients with cancer, while residual leukocytes and leukocyte-derived mediators may promote immunomodulation. Since leukoreduction is only able to relieve, but not eliminate TRIM, additional advanced techniques are expected to further minimize leukocyte numbers in RBC units. For patients with cancer receiving blood transfusions, fresh leukoreduced RBCs or even irradiated RBCs should be chosen where possible to avoid TRIM. However, in stored RBC supernatants, apart from leukocytes and the substances they release, EVs are also constantly secreted by RBCs in storage (77). EV numbers typically increase with longer storage durations and likely contribute to TRIM (77,78).

Previous studies have indicated that stored RBC-derived EVs are able to mediate TRIM and proinflammatory effects (78-80). RBC-derived EVs have been reported to inhibit the proliferation and activation of B cells and macrophages in a dose-dependent manner. Larger doses of EVs are able to stimulate macrophages to release IL-8, whilst significantly suppressing TNF-α (81). By contrast, exosomes from leukoreduced RBC units were observed to induce the secretion of proinflammatory cytokines and chemokines from peripheral blood mononuclear cells to strengthen T-cell responses (22). In addition, RBC-derived EVs may induce monocytes to secrete intercellular adhesion molecules and E-selectins to activate endothelial cells, thereby promoting proinflammatory and procoagulant effects (82). Numerous studies have previously indicated that systemic inflammation is an independent predictor of recurrence of breast cancer (83), pancreatic cancer (84), non-small-cell lung cancer (85) and colorectal cancer (86,87).

4. Biogenesis and characterization of stored RBC-derived EVs

With prolonged storage durations, the number of RBC-derived EVs gradually increases (88-90). Among these EVs, the number of small EVs (sEVs) with diameters <200 nm is greater than that of large EVs (lEVs) with diameters >200 nm (91). Leukoreduction may significantly reduce the quantity of EV in the RBC products (92). A variety of leukoreduction methods may confer different effects on the size and quantity of EVs in the final RBC transfusion pack (92). Typically, two primary methods are used to prepare leukoreduced packed RBC units, namely whole-blood filtration and red-cell filtration, the difference of which is in the time of leukoreduction (93). Whole-blood filtration involves the removal of leukocytes using a leukocyte depletion filter prior to its preparation into various leukoreduced blood products (93). By contrast, red-cell filtration first separates the majority of the plasma, platelets and leukocytes from the whole blood by centrifugation before RBC concentrates are prepared, from which leukocytes are subsequently removed using the leukocyte depletion filter to obtain the final product of leukoreduced RBC units (93). Therefore, the total number of EVs and the specific number of residual cell-derived EVs are both smaller in RBC concentrates collected through red-cell filtration (77,88,91). In particular, RBC products from B-type blood, compared with those from other blood types, have higher numbers of RBC-derived EV but lower residual platelet-derived EV numbers (94). The cause of this remains unknown and therefore warrants further investigation. In addition, RBC EV numbers have been reported to increase if certain filter types are used, including MacoPharma-LCRD2 for red-cell filtration and Fresenius-T2975 for whole-blood filtration, or if the RBC products were prepared on day 2 after blood collection (94). None of these findings were indicated to be associated with the sex or age of the donors (94).

Biogenesis of EVs. Due to metabolite accumulation/depletion and oxidative damage, the cytoskeleton of stored RBCs is damaged, such that the morphology of RBCs changes from the biconcave disc cell shape to echinocytes (95-99). Lipids and proteins carried within EVs may be released from the membrane, leading to reduced RBC deformation (95-99). The formation of RBC EVs is associated with changes in the phospholipid profile in the RBC membrane, particularly PS (100). Under physiological conditions, PS is exclusively present in the inner leaflet of the RBC membrane, which is regulated by three transporting enzymes, flippase (transporting PS inwards), scramblase (transporting PS bidirectionally) and floppase (transporting PS outward) (100). Exposed PS is typically the signal of RBC apoptosis and eryptosis (100,101), which is mainly mediated by flippase and scramblase (101-103). As the storage duration increases, the concentration of K^+ outside the cell also increases (100). At the same time, inside the erythrocytes, reductions in ATP concentration inhibit flippase, whilst decreases in membrane cholesterol levels lead to an increase in scramblase activity, which translocation of PS from the inner RBC membrane to the cell surface to form EVs (Fig. 1) (97,100,101). In addition, proteomic analysis of stored RBCs previously revealed an increase in Hb binding to the membrane and the aggregation

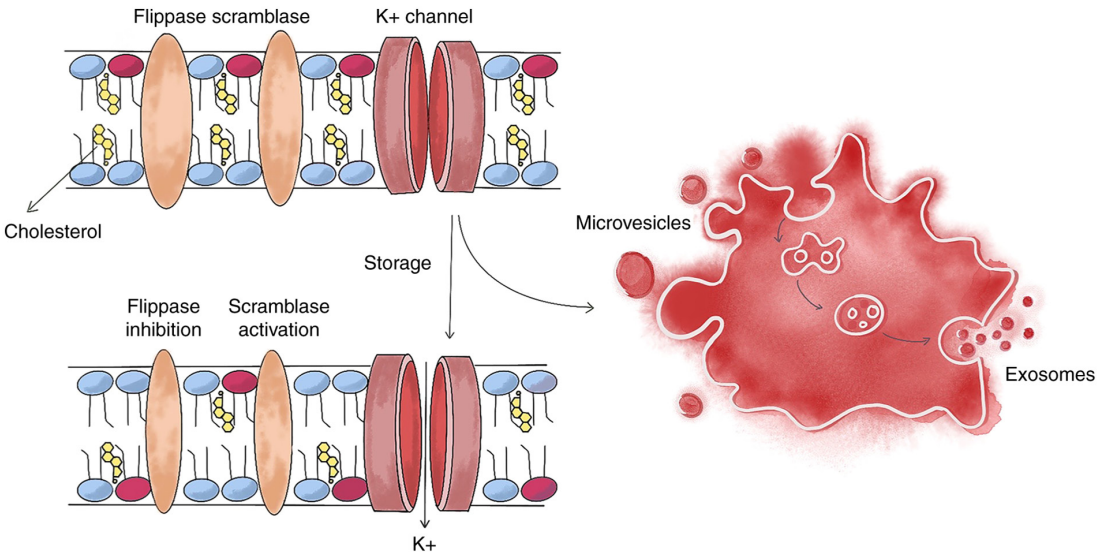

Figure 1. Lipid composition of RBCs. RBCs undergo storage lesions during storage, whereby cell membranes are damaged and extracellular vesicles are released. RBCs, red blood cells.

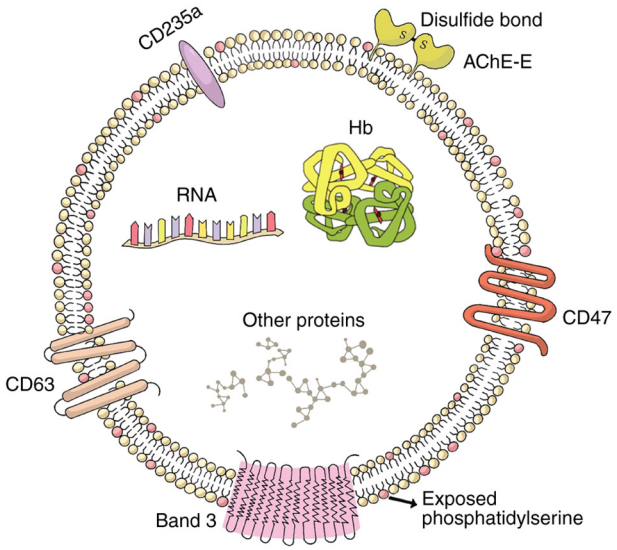

Figure 2. Biomarkers and contents contained within RBC-derived extracellular vesicles. Hb, hemoglobin; AchE-E, acetylcholinesterase-erythrocytes.

and degradation of integral membrane protein band 3, which is an indication of membrane remodeling during storage (25). The affinity between the denatured Hb and integral membrane protein band 3 may promote the binding of IgG and senescence antigens originating from band 3, triggering the formation of EVs (25).

During the process of RBC storage, since PS is increasingly exposed to the EVs, the majority of EVs are PS-positive (100). PS on the surface of RBCs is a recognition signal for macrophages, which increases the osmotic fragility of RBCs (104,105). Under oxidative stress, RBC-derived EVs contain highly oxidized, dysfunctional Hb (HbChr) (106). EVs released during RBC storage contain lipid raft proteins, oxidative or reactive signaling components associated with aging RBCs (23,107). RBCs transfer exposed PS, HbChr and damaged membrane components into EVs, which postpones the removal of healthy RBCs (23,106,108). Therefore, the generation of EVs may result from the auto-protective mechanism of RBCs (107,109).

Accumulating evidence suggests that after Ca^{2+} is added, exposure of PS on the surface of RBCs and the formation of EVs are associated with increased Ca^{2+} levels inside the cell (102,103,110). Although citrates in the RBC preservation solution may chelate Ca^{2+} to a reduce Ca^{2+} in the plasma, it has been reported that EDTA, heparin and citrates are unable to completely chelate Ca^{2+} in extracellular medium, such that Ca^{2+} may be released by platelets and leukocytes following cell death to be taken up by RBCs (111). Thus, Cloos *et al* (111) proposed four consecutive events in the biogenesis of stored RBC-derived EVs, namely cholesterol domain decrease, oxidative stress, sphingomyelin/sphingomyelinase/ceramide/Ca^{2+} alteration and PS exposure. However, Sudnitsyna *et al* (106) documented that the oxidative stress process, whereby Hb is oxidized to HbChr, is the primary trigger of RBC transformation and formation of EVs independent of intracellular Ca^{2+} levels (106). Therefore, the association between changes in the intracellular Ca^{2+} levels and the formation of EVs remains controversial and requires further investigation.

Characterization. Based on differences in their biogenesis, EVs may be assigned to the following three categories: Cup-shaped exosomes originating from the endosomal network; microvesicles that are constantly undergoing cycles of budding and fission at the plasma membrane; and apoptotic bodies released from apoptotic cells (112,113). The majority of RBC-derived EVs are <1,000 nm in diameter and most of them are ~200 nm (22,93,113). These EVs are primarily comprised of the following two types: sEVs (50-200 nm) and lEVs (150-300 nm) (22,26,113). Transmission electron microscopy images revealed the ball shape of stored RBC-derived EVs, but a certain degree of heterogeneity in terms of form and size (16,114). Particle sizes and concentrations of EVs may be measured using flow cytometry, tunable resistive pulse sensing (TRPS), dynamic light scattering (DLS) and

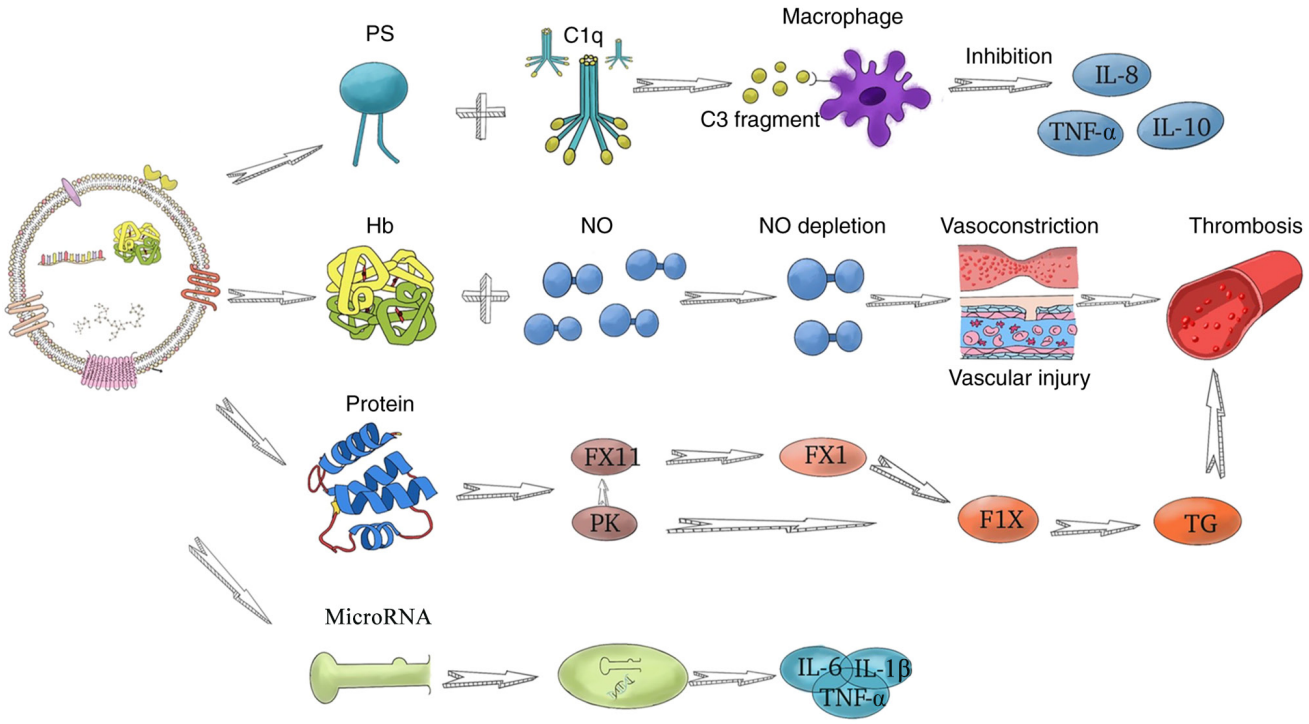

Figure 3. Mechanism of stored RBC-derived EVs in TRIM in patients with cancer receiving transfusions. PS on the surface of RBC-derived EVs are able to bind to C1q in the blood to activate the classical pathway of complement and inhibit immune cells from releasing a variety of immune factors, such as TNF-α. Hb binding to EVs may interact with NO, leading to the consumption of NO, contraction of blood vessels, formation of thrombosis and increased risk of transfusion-induced inflammation. Proteins from RBC EVs are able to activate factor IX through two independent pathways, the classical FXIIa/FXI/FIX pathway and direct kallikrein to mediate thrombosis. RBC-derived EVs are able to carry RNAs, especially microRNAs, which may influence various biological processes, including signal transduction and nucleotide metabolism to mediate intercellular communication. RBC, red blood cell; NO, nitric oxide; PS, phosphatidylserine; C1q, complement C1q; Hb, hemoglobin; NO, nitric oxide; FXII, coagulation factor XII; FXI, coagulation factor XI; FIX, coagulation factor IX; TG, thrombin generation; PK, pre-kallikrein; TRIM, transfusion-related immunomodulation; EV, extracellular vesicle.

nanoparticle tracking analysis (88,115). DLS was previously used to detect significant increases in the average sizes of EVs in stored RBCs, whilst TRPS was used to reveal significant decreases (93). This contrast may be explained by the tendency of DLS to bias the analysis towards the detection of larger particles (116).

According to recommendations from The Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018) guidelines, ≥3 positive protein markers are required for the characterization of EVs, including ≥1 transmembrane/lipid-bound protein, a cytosolic protein and ≥1 negative protein marker (117). For transmembrane proteins, the tetraspanin CD63 (117) and the multi-pass membrane protein CD47 (94) may be used to confirm the presence of the lipid bilayer in EVs. By contrast, CD235a (22,81,118) or acetylcholinesterase-erythrocytes (117) may be used as erythrocyte-specific markers. Hb, a cytosolic protein unique to RBCs, may be wrapped in the lipid-bilayer and then released with EVs (Fig. 2) (16). In addition, apolipoproteins A1/2 and B (16) or albumin (117) may be used as a negative control to assess the purity of EVs. Previous studies have indicated that EVs from leukoreduced RBC products are primarily of RBC origin (108), which may be contaminated in storage by platelets and other cell types instead of leukocytes (22). Following the publication of the MISEV2018 guidelines, a number of studies that used only one or two protein markers for the characterization of stored RBCs persisted, which should have been performed in accordance with unified standards (77,93,94). To date, there has been no consensus on which protein markers should be used to distinguish lEVs and microvesicles from sEVs and exosomes, since EVs may be produced using different centrifugation methods from different cell types (117). Using CD63, Danesh *et al* (22) differentiated exosomes from sEVs derived from RBCs and proposed that exosomes are CD63-positive. However, their results require further verification.

5. Mechanism of stored RBC-derived EVs in TRIM in patients with cancer receiving transfusions

Since they may carry a variety of lipids, proteins and nucleic acids, EVs are able to mediate cell-to-cell communication to regulate cellular processes, including inflammation, immune signaling and angiogenesis (Fig. 3) (119). EVs may also be potentially used as cancer biomarkers (120,121). RBC-derived EVs are able to bind to C1q in the blood, activate the classical complement pathway and suppress the function of both macrophages and the immune system (81). Such binding may be mediated by PS on the surfaces of RBCs (65). RBC-derived microvesicles that contain PS were observed to increase systemic inflammation in mice by the thrombin-dependent activation of complement (122). In addition, proteins from RBC EVs may activate factor IX through two independent pathways, namely the classical coagulation factor (F)XIIa/FXI/FIX pathway and the direct kallikrein pathway, to mediate inflammatory and/or thrombotic activities (114). During storage,

Hb is released from RBCs into the preservation medium in the form of cell-free Hb and microparticles as a result of storage lesions, such that a longer storage duration leads to a higher concentration (90,123). After RBC transfusion, cell-free Hb and Hb binding to EVs interact with nitric oxide (NO) (124), leading to the contraction of blood vessels, formation of thrombosis and increased risk of transfusion-induced inflammation (125). Such reactions were also observed to be 1,000-fold faster compared with those mediated by complete RBCs (90,123). NO, on the one hand, may be cytotoxic and able to induce apoptosis of cells as an anti-tumor agent, but on the other hand, it may promote angiogenesis and cancer metastasis as an oncogenic agent, which associates it with cancer (126). RBC-derived EVs may also carry several types of RNA to mediate communication between cells (26,127). It has been previously reported that the highest quantity of microRNA (miR)-451a (26) from RBC-derived EVs is able to regulate innate immunity, inflammatory responses and immune functions (128,129). Reduced expression of miR-451a was indicated to upregulate the expression of macrophage migration inhibitory factor in breast cancer (130) and that of phosphomannomutase-2 in renal cell carcinoma (131), both of which are associated with increased metastatic and invasive abilities of tumor cells (131,132).

It should be noted that a number of studies have also suggested that monocyte suppression is not only mediated by EVs separated from RBC units alone, but other potential soluble mediators, such as miRs (118). Residual platelet-derived EVs in RBC products have been previously detected (94), which are potent mediators of inflammation *in vitro* (133). It was previously reported that although leukoreduction alone was not able to reduce TRIM, leukoreduction and radiation with γ-rays together was able to, suggesting that γ-rays may enhance the impact of leukoreduction on alloimmunity (134). After leukoreduction through leukocyte filtration, leukocytes of $5x10^6$ units typically persist, meaning that the continuous existence of filtrate leukocytes remains accountable for TRIM (12). Mechanistically, γ-rays act on the nuclei of white blood cells to induce apoptosis, thereby reducing microchimerism and allosensitization (135). In addition, after transfusion with leukoreduced and γ-irradiated RBCs, regulatory T cells exhibited reduced activity, which in turn reduced the extent of immunosuppression in the body (134). Therefore, TRIM in patients with cancer may be concluded to be due to a combined action of factors, including residual leukocytes, residual platelets and EVs. Stored RBC-derived EVs may inhibit the proliferation and activation of immune cells through multiple mechanisms. Thus, attention should be paid to their roles in TRIM. RBC transfusions enable the entry of large quantities of immunosuppressive EVs into the body. Therefore, the potential negative impact of RBC transfusions on patients with cancer should be taken into full consideration.

6. Outlook

An increasing number of studies have indicated that RBCs and immune cells interact with each other. RBCs contain a variety of immunoregulatory factors, suggesting, to a certain extent, that RBCs themselves may be involved in TRIM. Characterization of RBC EVs is the focus of the majority of recent investigations, though it remains unclear whether Ca^{2+} serves a role in the generation of RBC EVs. To date, studies into the immunomodulatory role of stored RBC EVs have been limited to *in vitro* studies and animal models. Further clinical studies are required to investigate the full impact of RBC EVs on the human immune system. In addition, it remains unknown how stored RBC EVs exert their immunomodulatory roles, specifically what roles the proteins and RNAs they carry serve. The quantity of RBC EVs is affected by a variety of factors, which influences not only the quality of RBC products, but also the clinical outcomes of patients. In terms of the effects of external factors, including differences in filters and blood processing time, enhanced measures should be taken to control blood preparation procedures and to reduce the number of EVs in blood products.

RBC transfusion is a common therapeutic option for anemia in patients with advanced cancer. However, RBC transfusions are becoming increasingly associated with unfavorable prognoses in patients with malignancies. Therefore, on the basis of some guidelines (48,136), a number of recommendations has been proposed as precautionary measures: To reduce blood transfusion where possible when the normal Hb level is maintained; to apply autotransfusion and fresh RBC products if transfusions are necessary; to remove residual leukocytes and EVs from RBC products through leukocyte filtration; to use irradiated RBCs if possible; and to apply conservative blood transfusion strategies for patients with cancer (Hb ≤7-8 g/l).

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Students' Innovation and Entrepreneurship Training Program (grant no. 202010632069).

Availability of data and materials

Not applicable.

Authors' contributions

XYM wrote the manuscript. YXL and QLH drew the figures. HWZ, JW and YWH revised the paper. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Gantt CL: Red blood cells for patients with cancer. Lancet 2: 363, 1981.
2. Parmiani G, Fossati G and Della Porta G: The undefined relationship between tumor antigens and histocompatibility antigens on cancer cells. Ric Clin Lab 10: 481-492, 1980.
3. Jiang XB, Zhang LP, Wang YJ and Ma C: Research advance on clinical blood transfusion and tumor therapy. Zhongguo Shi Yan Xue Ye Xue Za Zhi 18: 1092-1095, 2010 (In Chinese).
4. Kenar G, Köksoy EB, Ürün Y and Utkan G: Prevalence, etiology and risk factors of anemia in patients with newly diagnosed cancer. Support Care Cancer 28: 5235-5242, 2020.
5. Owusu C, Cohen HJ, Feng T, Tew W, Mohile SG, Klepin HD, Gross CP, Gajra A, Lichtman SM and Hurria A; Cancer and Aging Research Group (CARG): Anemia and functional disability in older adults with cancer. J Natl Compr Canc Netw 13: 1233-1239, 2015.
6. Watkins T, Surowiecka MK and McCullough J: Transfusion indications for patients with cancer. Cancer Control 22: 38-46, 2015.
7. Dicato M, Plawny L and Diederich M: Anemia in cancer. Ann Oncol 21 (Suppl 7): vii167-vii172, 2010.
8. Shortt J, Polizzotto MN, Waters N, Borosak M, Moran M, Comande M, Devine A, Jolley DJ and Wood EM: Assessment of the urgency and deferability of transfusion to inform emergency blood planning and triage: The bloodhound prospective audit of red blood cell use. Transfusion 49: 2296-2303, 2009.
9. Tzounakas VL, Seghatchian J, Grouzi E, Kokoris S and Antonelou MH: Red blood cell transfusion in surgical cancer patients: Targets, risks, mechanistic understanding and further therapeutic opportunities. Transfus Apher Sci 56: 291-304, 2017.
10. Al-Refaie WB, Parsons HM, Markin A, Abrams J and Habermann EB: Blood transfusion and cancer surgery outcomes: A continued reason for concern. Surgery 152: 344-354, 2012.
11. Aguilar-Nascimento JE, Zampieri-Filho JP and Bordin JO: Implications of perioperative allogeneic red blood cell transfusion on the immune-inflammatory response. Hematol Transfus Cell Ther 43: 58-64, 2021.
12. Connor JP, O'Shea A, McCool K, Sampene E and Barroilhet LM: Peri-operative allogeneic blood transfusion is associated with poor overall survival in advanced epithelial ovarian cancer; potential impact of patient blood management on cancer outcomes. Gynecol Oncol 151: 294-298, 2018.
13. Goubran H, Sheridan D, Radosevic J, Burnouf T and Seghatchian J: Transfusion-related immunomodulation and cancer. Transfus Apher Sci 56: 336-340, 2017.
14. Remy KE, Hall MW, Cholette J, Juffermans NP, Nicol K, Doctor A, Blumberg N, Spinella PC, Norris PJ, Dahmer MK, *et al*: Mechanisms of red blood cell transfusion-related immunomodulation. Transfusion 58: 804-815, 2018.
15. D'Alessandro A, Kriebardis AG, Rinalducci S, Antonelou MH, Hansen KC, Papassideri IS and Zolla L: An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. Transfusion 55: 205-219, 2015.
16. Laurén E, Tigistu-Sahle F, Valkonen S, Westberg M, Valkeajärvi A, Eronen J, Siljander P, Pettilä V, Käkelä R, Laitinen S and Kerkelä E: Phospholipid composition of packed red blood cells and that of extracellular vesicles show a high resemblance and stability during storage. Biochim Biophys Acta Mol Cell Biol Lipids 1863: 1-8, 2018.
17. Antonelou MH and Seghatchian J: Insights into red blood cell storage lesion: Toward a new appreciation. Transfus Apher Sci 55: 292-301, 2016.
18. Hoehn RS, Jernigan PL, Chang AL, Edwards MJ and Pritts TA: Molecular mechanisms of erythrocyte aging. Biol Chem 396: 621-631, 2015.
19. Nieuwland R, Falcon-Perez JM, Soekmadji C, Boilard E, Carter D and Buzas EI: Essentials of extracellular vesicles: Posters on basic and clinical aspects of extracellular vesicles. J Extracell Vesicles 7: 1548234, 2018.
20. van der Pol E, Böing AN, Harrison P, Sturk A and Nieuwland R: Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 64: 676-705, 2012.
21. van Niel G, D'Angelo G and Raposo G: Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 19: 213-228, 2018.
22. Danesh A, Inglis HC, Jackman RP, Wu S, Deng X, Muench MO, Heitman JW and Norris PJ: Exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses in vitro. Blood 123: 687-696, 2014.
23. Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH and Papassideri IS: RBC-derived vesicles during storage: Ultrastructure, protein composition, oxidation, and signaling components. Transfusion 48: 1943-1953, 2008.
24. Azarov I, Liu C, Reynolds H, Tsekouras Z, Lee JS, Gladwin MT and Kim-Shapiro DB: Mechanisms of slower nitric oxide uptake by red blood cells and other hemoglobin-containing vesicles. J Biol Chem 286: 33567-33579, 2011.
25. Bosman GJ, Lasonder E, Luten M, Roerdinkholder-Stoelwinder B, Novotný VM, Bos H and De Grip WJ: The proteome of red cell membranes and vesicles during storage in blood bank conditions. Transfusion 48: 827-835, 2008.
26. Huang H, Zhu J, Fan L, Lin Q, Fu D, Wei B and Wei S: MicroRNA profiling of exosomes derived from red blood cell units: Implications in transfusion-related immunomodulation. Biomed Res Int 2019: 2045915, 2019.
27. Saas P, Angelot F, Bardiaux L, Seilles E, Garnache-Ottou F and Perruche S: Phosphatidylserine-expressing cell by-products in transfusion: A pro-inflammatory or an anti-inflammatory effect? Transfus Clin Biol 19: 90-97, 2012.
28. Deeb AP, Aquina CT, Monson JRT, Blumberg N, Becerra AZ and Fleming FJ: Allogeneic leukocyte-reduced red blood cell transfusion is associated with postoperative infectious complications and cancer recurrence after colon cancer resection. Dig Surg 37: 163-170, 2020.
29. Tamini N, Deghi G, Gianotti L, Braga M and Nespoli L: Colon cancer surgery: Does preoperative blood transfusion influence short-term postoperative outcomes? J Invest Surg 34: 974-978, 2021.
30. Qiu L, Wang DR, Zhang XY, Gao S, Li XX, Sun GP and Lu XB: Impact of perioperative blood transfusion on immune function and prognosis in colorectal cancer patients. Transfus Apher Sci 54: 235-241, 2016.
31. Acheson AG, Brookes MJ and Spahn DR: Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: A systematic review and meta-analysis. Ann Surg 256: 235-244, 2012.
32. Liu X, Ma M, Huang H and Wang Y: Effect of perioperative blood transfusion on prognosis of patients with gastric cancer: A retrospective analysis of a single center database. BMC Cancer 18: 649, 2018.
33. Benson D and Barnett CC Jr: Perioperative blood transfusions promote pancreas cancer progression. J Surg Res 166: 275-279, 2011.
34. Churchhouse AM, Mathews TJ, McBride OM and Dunning J: Does blood transfusion increase the chance of recurrence in patients undergoing surgery for lung cancer? Interact Cardiovasc Thorac Surg 14: 85-90, 2012.
35. Seon DY, Kwak C, Kim HH, Ku JH and Kim HS: Impact of perioperative blood transfusion on oncologic outcomes in patients with nonmetastatic renal cell carcinoma treated with curative nephrectomy: A retrospective analysis of a large, single-institutional cohort. Investig Clin Urol 61: 136-145, 2020.
36. Nizri E, Kusamura S, Fallabrino G, Guaglio M, Baratti D and Deraco M: Dose-dependent effect of red blood cells transfusion on perioperative and long-term outcomes in peritoneal surface malignancies treated with cytoreduction and HIPEC. Ann Surg Oncol 25: 3264-3270, 2018.
37. Cata JP, Wang H, Gottumukkala V, Reuben J and Sessler DI: Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions. Br J Anaesth 110: 690-701, 2013.
38. Wu HL, Tai YH, Lin SP, Chan MY, Chen HH and Chang KY: The impact of blood transfusion on recurrence and mortality following colorectal cancer resection: A propensity score analysis of 4,030 patients. Sci Rep 8: 13345, 2018.
39. Grasso M, Pacella G, Sangiuliano N, De Palma M and Puzziello A: Gastric cancer surgery: clinical outcomes and prognosis are influenced by perioperative blood transfusions. Updates Surg 71: 439-443, 2019.
40. Gunka I, Dostalik J, Martinek L, Gunkova P and Mazur M: Impact of blood transfusions on survival and recurrence in colorectal cancer surgery. Indian J Surg 75: 94-101, 2013.
41. Halabi WJ, Jafari MD, Nguyen VQ, Carmichael JC, Mills S, Pigazzi A and Stamos MJ: Blood transfusions in colorectal cancer surgery: Incidence, outcomes, and predictive factors: An American college of surgeons national surgical quality improvement program analysis. Am J Surg 206: 1024-1033, 2013.

42. Baguena G, Pellino G, Frasson M, Escrig J, Marinello F, Espí A, García-Granero A, Roselló S, Cervantes A and García-Granero E: Impact of perioperative transfusions and sepsis on long-term oncologic outcomes after curative colon cancer resection. A retrospective analysis of a prospective database. Gastroenterol Hepatol 43: 63-72, 2020 (In English, Spanish).
43. Tarantino I, Ukegjini K, Warschkow R, Schmied BM, Steffen T, Ulrich A and Müller SA: Blood transfusion does not adversely affect survival after elective colon cancer resection: A propensity score analysis. Langenbecks Arch Surg 398: 841-849, 2013.
44. Hunsicker O, Gericke S, Graw JA, Krannich A, Boemke W, Meyer O, Braicu I, Spies C, Sehouli J, Pruß A and Feldheiser A: Transfusion of red blood cells does not impact progression-free and overall survival after surgery for ovarian cancer. Transfusion 59: 3589-3600, 2019.
45. Zaw AS, Kantharajanna SB, Maharajan K, Tan B, Vellayappan B and Kumar N: Perioperative blood transfusion: Does it influence survival and cancer progression in metastatic spine tumor surgery? Transfusion 57: 440-450, 2017.
46. Chang CC, Lee TC, Su MJ, Lin HC, Cheng FY, Chen YT, Yen TH and Chu FY: Transfusion-associated adverse reactions (TAARs) and cytokine accumulations in the stored blood components: The impact of prestorage versus poststorage leukoreduction. Oncotarget 9: 4385-4394, 2017.
47. Chalfin HJ, Liu JJ, Gandhi N, Feng Z, Johnson D, Netto GJ, Drake CG, Hahn NM, Schoenberg MP, Trock BJ, *et al*: Blood transfusion is associated with increased perioperative morbidity and adverse oncologic outcomes in bladder cancer patients receiving neoadjuvant chemotherapy and radical cystectomy. Ann Surg Oncol 23: 2715-2722, 2016.
48. Carson JL, Guyatt G, Heddle NM, Grossman BJ, Cohn CS, Fung MK, Gernsheimer T, Holcomb JB, Kaplan LJ, Katz LM, *et al*: Clinical practice guidelines from the AABB: Red blood cell transfusion thresholds and storage. JAMA 316: 2025-2035, 2016.
49. Alkhalid Y, Lagman C, Sheppard JP, Nguyen T, Prashant GN, Ziman AF and Yang I: Restrictive transfusion threshold is safe in high-risk patients undergoing brain tumor surgery. Clin Neurol Neurosurg 163: 103-107, 2017.
50. Boone JD, Kim KH, Marques M and Straughn JM: Compliance rates and outcomes associated with a restrictive transfusion policy in gynecologic oncology patients. Gynecol Oncol 132: 227-230, 2014.
51. Syan-Bhanvadia S, Drangsholt S, Shah S, Cai J, Miranda G, Djaladat H and Daneshmand S: Restrictive transfusion in radical cystectomy is safe. Urol Oncol 35: 528.e15-528.e21, 2017.
52. Wehry J, Agle S, Philips P, Cannon R, Scoggins CR, Puffer L, McMasters KM and Martin RC: Restrictive blood transfusion protocol in malignant upper gastrointestinal and pancreatic resections patients reduces blood transfusions with no increase in patient morbidity. Am J Surg 210: 1197-1205, 2015.
53. Prescott LS, Taylor JS, Lopez-Olivo MA, Munsell MF, VonVille HM, Lairson DR and Bodurka DC: How low should we go: A systematic review and meta-analysis of the impact of restrictive red blood cell transfusion strategies in oncology. Cancer Treat Rev 46: 1-8, 2016.
54. Ozben V, Stocchi L, Ashburn J, Liu X and Gorgun E: Impact of a restrictive vs liberal transfusion strategy on anastomotic leakage and infectious complications after restorative surgery for rectal cancer. Colorectal Dis 19: 772-780, 2017.
55. Bergamin FS, Almeida JP, Landoni G, Galas FRBG, Fukushima JT, Fominskiy E, Park CHL, Osawa EA, Diz MPE, Oliveira GQ, *et al*: Liberal versus restrictive transfusion strategy in critically Ill oncologic patients: The transfusion requirements in critically Ill oncologic patient randomized controlled trial. Crit Care Med 45: 766-773, 2017.
56. Crawford TM, Andersen CC and Stark MJ: Effect of repeat transfusion exposure on plasma cytokine and markers of endothelial activation in the extremely preterm neonate. Transfusion 60: 2217-2224, 2020.
57. Muszynski JA, Spinella PC, Cholette JM, Acker JP, Hall MW, Juffermans NP, Kelly DP, Blumberg N, Nicol K, Liedel J, *et al*: Transfusion-related immunomodulation: Review of the literature and implications for pediatric critical illness. Transfusion 57: 195-206, 2017.
58. Opelz G, Sengar DP, Mickey MR and Terasaki PI: Effect of blood transfusions on subsequent kidney transplants. Transplant Proc 5: 253-259, 1973.
59. Carpenter CB: Blood transfusion effects in kidney transplantation. Yale J Biol Med 63: 435-443, 1990.
60. Abdolmohammadi K, Mahmoudi T, Jafari-Koshki T, Hassan ZM and Pourfathollah AA: Immunomodulatory effects of blood transfusion on tumor size, metastasis, and survival in experimental fibrosarcoma. Indian J Hematol Blood Transfus 34: 697-702, 2018.
61. Atzil S, Arad M, Glasner A, Abiri N, Avraham R, Greenfeld K, Rosenne E, Beilin B and Ben-Eliyahu S: Blood transfusion promotes cancer progression: A critical role for aged erythrocytes. Anesthesiology 109: 989-997, 2008.
62. Sugita S, Sasaki A, Iwaki K, Uchida H, Kai S, Shibata K, Ohta M and Kitano S: Prognosis and postoperative lymphocyte count in patients with hepatocellular carcinoma who received intraoperative allogenic blood transfusion: A retrospective study. Eur J Surg Oncol 34: 339-345, 2008.
63. Chen G, Zhang FJ, Gong M and Yan M: Effect of perioperative autologous versus allogeneic blood transfusion on the immune system in gastric cancer patients. J Zhejiang Univ Sci B 8: 560-565, 2007.
64. Tao CJ, Chen YY, Jiang F, Feng XL, Jin QF, Jin T, Piao YF and Chen XZ: A prognostic model combining CD4/CD8 ratio and N stage predicts the risk of distant metastasis for patients with nasopharyngeal carcinoma treated by intensity modulated radiotherapy. Oncotarget 7: 46653-46661, 2016.
65. Sparrow RL: Red blood cell storage and transfusion-related immunomodulation. Blood Transfus 8 (Suppl 3): s26-s30, 2010.
66. Clark DA, Gorczynski RM and Blajchman MA: Transfusion-related immunomodulation due to peripheral blood dendritic cells expressing the CD200 tolerance signaling molecule and alloantigen. Transfusion 48: 814-821, 2008.
67. Teicher BA: Transforming growth factor-beta and the immune response to malignant disease. Clin Cancer Res 13: 6247-6251, 2007.
68. Pinheiro MK, Tamagne M, Elayeb R, Andrieu M, Pirenne F and Vingert B: Blood microparticles are a component of immune modulation in red blood cell transfusion. Eur J Immunol 50: 1237-1240, 2020.
69. Ghio M, Contini P, Ubezio G, Mazzei C, Puppo F and Indiveri F: Immunomodulatory effects of blood transfusions: The synergic role of soluble HLA Class I free heavy-chain molecules detectable in blood components. Transfusion 48: 1591-1597, 2008.
70. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S and Puppo F: Soluble HLA-A,-B,-C and -G molecules induce apoptosis in T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol 33: 125-134, 2003.
71. Ottonello L, Ghio M, Contini P, Bertolotto M, Bianchi G, Montecucco F, Colonna M, Mazzei C, Dallegri F and Indiveri F: Nonleukoreduced red blood cell transfusion induces a sustained inhibition of neutrophil chemotaxis by stimulating in vivo production of transforming growth factor-beta1 by neutrophils: Role of the immunoglobulinlike transcript 1, sFasL, and sHLA-I. Transfusion 47: 1395-1404, 2007.
72. Baumgartner JM, Silliman CC, Moore EE, Banerjee A and McCarter MD: Stored red blood cell transfusion induces regulatory T cells. J Am Coll Surg 208: 110-119, 2009.
73. Zhuang Y, Zhang T, Wei C, Pan JC, Wang SF, Zhang AQ and Wang DQ: Effect of leukoreduction on tumor-associated cytokine accumutation in supernatant of stored packed red cells and its effect on tumor cell proliferation in vitro. Zhongguo Shi Yan Xue Ye Xue Za Zhi 23: 217-221, 2015 (In Chinese).
74. Baumgartner JM, Nydam TL, Clarke JH, Banerjee A, Silliman CC and McCarter MD: Red blood cell supernatant potentiates LPS-induced proinflammatory cytokine response from peripheral blood mononuclear cells. J Interferon Cytokine Res 29: 333-338, 2009.
75. Long K, Meier C, Ward M, Williams D, Woodward J and Bernard A: Immunologic profiles of red blood cells using in vitro models of transfusion. J Surg Res 184: 567-571, 2013.
76. Ohue Y and Nishikawa H: Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci 110: 2080-2089, 2019.
77. Almizraq RJ, Holovati JL and Acker JP: Characteristics of extracellular vesicles in red blood concentrates change with storage time and blood manufacturing method. Transfus Med Hemother 45: 185-193, 2018.
78. Menocha S and Muszynski JA: Transfusion-related immune modulation: Functional consequence of extracellular vesicles? Transfusion 59: 3553-3555, 2019.
79. Almizraq RJ, Seghatchian J and Acker JP: Extracellular vesicles in transfusion-related immunomodulation and the role of blood component manufacturing. Transfus Apher Sci 55: 281-291, 2016.

80. Sut C, Tariket S, Chou ML, Garraud O, Laradi S, Hamzeh-Cognasse H, Seghatchian J, Burnouf T and Cognasse F: Duration of red blood cell storage and inflammatory marker generation. Blood Transfus 15: 145-152, 2017.
81. Sadallah S, Eken C and Schifferli JA: Erythrocyte-derived ectosomes have immunosuppressive properties. J Leukoc Biol 84: 1316-1325, 2008.
82. Straat M, van Hezel ME, Böing A, Tuip-De Boer A, Weber N, Nieuwland R, van Bruggen R and Juffermans NP: Monocyte-mediated activation of endothelial cells occurs only after binding to extracellular vesicles from red blood cell products, a process mediated by β-integrin. Transfusion 56: 3012-3020, 2016.
83. Cole SW: Chronic inflammation and breast cancer recurrence. J Clin Oncol 27: 3418-3419, 2009.
84. Nakagawa K, Sho M, Akahori T, Nagai M, Nakamura K, Takagi T, Tanaka T, Nishiofuku H, Ohbayashi C, Kichikawa K and Ikeda N: Significance of the inflammation-based prognostic score in recurrent pancreatic cancer. Pancreatology 19: 722-728, 2019.
85. Guo D, Zhang J, Jing W, Liu J, Zhu H, Fu L, Li M, Kong L, Yue J and Yu J: Prognostic value of systemic immune-inflammation index in patients with advanced non-small-cell lung cancer. Future Oncol 14: 2643-2650, 2018.
86. Matsubara D, Arita T, Nakanishi M, Kuriu Y, Murayama Y, Kudou M, Konishi H, Komatsu S, Shiozaki A and Otsuji E: The impact of postoperative inflammation on recurrence in patients with colorectal cancer. Int J Clin Oncol 25: 602-613, 2020.
87. Yoshida D, Minami K, Sugiyama M, Ota M, Ikebe M, Morita M, Matsukuma A and Toh Y: Prognostic impact of the neutrophil-to-lymphocyte ratio in stage I-II rectal cancer patients. J Surg Res 245: 281-287, 2020.
88. Acker JP, Almizraq RJ, Millar D and Maurer-Spurej E: Screening of red blood cells for extracellular vesicle content as a product quality indicator. Transfusion 58: 2217-2226, 2018.
89. Almizraq RJ, Seghatchian J, Holovati JL and Acker JP: Extracellular vesicle characteristics in stored red blood cell concentrates are influenced by the method of detection. Transfus Apher Sci 56: 254-260, 2017.
90. Donadee C, Raat NJ, Kanias T, Tejero J, Lee JS, Kelley EE, Zhao X, Liu C, Reynolds H, Azarov I, *et al*: Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 124: 465-476, 2011.
91. Almizraq RJ, Norris PJ, Inglis H, Menocha S, Wirtz MR, Juffermans N, Pandey S, Spinella PC, Acker JP and Muszynski JA: Blood manufacturing methods affect red blood cell product characteristics and immunomodulatory activity. Blood Adv 2: 2296-2306, 2018.
92. Richter JR, Sutton JM, Hexley P, Johannigman TA, Lentsch AB and Pritts TA: Leukoreduction of packed red blood cells attenuates proinflammatory properties of storage-derived microvesicles. J Surg Res 223: 128-135, 2018.
93. Bicalho B, Pereira AS and Acker JP: Buffy coat (top/bottom)- and whole-blood filtration (top/top)-produced red cell concentrates differ in size of extracellular vesicles. Vox Sang 109: 214-220, 2015.
94. Gamonet C, Desmarets M, Mourey G, Biichle S, Aupet S, Laheurte C, François A, Resch E, Bigey F, Binda D, *et al*: Processing methods and storage duration impact extracellular vesicle counts in red blood cell units. Blood Adv 4: 5527-5539, 2020.
95. Yoshida T, Prudent M and D'Alessandro A: Red blood cell storage lesion: Causes and potential clinical consequences. Blood Transfus 17: 27-52, 2019.
96. Kozlova E, Chernysh A, Moroz V, Kozlov A, Sergunova V, Sherstyukova E and Gudkova O: Two-step process of cytoskeletal structural damage during long-term storage of packed red blood cells. Blood Transfus 19: 124-134, 2021.
97. Kaczmarska M, Grosicki M, Bulat K, Mardyla M, Szczesny-Malysiak E, Blat A, Dybas J, Sacha T and Marzec KM: Temporal sequence of the human RBCs' vesiculation observed in nano-scale with application of AFM and complementary techniques. Nanomedicine 28: 102221, 2020.
98. Bicalho B, Holovati JL and Acker JP: Phospholipidomics reveals differences in glycerophosphoserine profiles of hypothermically stored red blood cells and microvesicles. Biochim Biophys Acta 1828: 317-326, 2013.
99. McVey MJ, Kuebler WM, Orbach A, Arbell D, Zelig O, Barshtein G and Yedgar S: Reduced deformability of stored red blood cells is associated with generation of extracellular vesicles. Transfus Apher Sci 59: 102851, 2020.
100. Burger P, Kostova E, Bloem E, Hilarius-Stokman P, Meijer AB, van den Berg TK, Verhoeven AJ, de Korte D and van Bruggen R: Potassium leakage primes stored erythrocytes for phosphatidylserine exposure and shedding of pro-coagulant vesicles. Br J Haematol 160: 377-386, 2013.
101. Arashiki N and Takakuwa Y: Maintenance and regulation of asymmetric phospholipid distribution in human erythrocyte membranes: Implications for erythrocyte functions. Curr Opin Hematol 24: 167-172, 2017.
102. Wesseling MC, Wagner-Britz L, Nguyen DB, Asanidze S, Mutua J, Mohamed N, Hanf B, Ghashghaeinia M, Kaestner L and Bernhardt I: Novel insights in the regulation of phosphatidylserine exposure in human red blood cells. Cell Physiol Biochem 39: 1941-1954, 2016.
103. Nguyen DB, Wagner-Britz L, Maia S, Steffen P, Wagner C, Kaestner L and Bernhardt I: Regulation of phosphatidylserine exposure in red blood cells. Cell Physiol Biochem 28: 847-856, 2011.
104. Tanaka Y and Schroit AJ: Insertion of fluorescent phosphatidylserine into the plasma membrane of red blood cells. Recognition by autologous macrophages. J Biol Chem 258: 11335-11343, 1983.
105. Orbach A, Zelig O, Yedgar S and Barshtein G: Biophysical and biochemical markers of red blood cell fragility. Transfus Med Hemother 44: 183-187, 2017.
106. Sudnitsyna J, Skverchinskaya E, Dobrylko I, Nikitina E, Gambaryan S and Mindukshev I: Microvesicle formation induced by oxidative stress in human erythrocytes. Antioxidants (Basel) 9: 929, 2020.
107. Prudent M, Delobel J, Hübner A, Benay C, Lion N and Tissot JD: Proteomics of stored red blood cell membrane and storage-induced microvesicles reveals the association of flotillin-2 with band 3 complexes. Front Physiol 9: 421, 2018.
108. Willekens FL, Werre JM, Groenen-Döpp YA, Roerdinkholder-Stoelwinder B, de Pauw B and Bosman GJ: Erythrocyte vesiculation: A self-protective mechanism? Br J Haematol 141: 549-556, 2008.
109. Tissot JD, Rubin O and Canellini G: Analysis and clinical relevance of microparticles from red blood cells. Curr Opin Hematol 17: 571-577, 2010.
110. Wagner-Britz L, Wang J, Kaestner L and Bernhardt I: Protein kinase Cα and P-type Ca channel CaV2.1 in red blood cell calcium signalling. Cell Physiol Biochem 31: 883-891, 2013.
111. Cloos AS, Ghodsi M, Stommen A, Vanderroost J, Dauguet N, Pollet H, D'Auria L, Mignolet E, Larondelle Y, Terrasi R, *et al*: Interplay between plasma membrane lipid alteration, oxidative stress and calcium-based mechanism for extracellular vesicle biogenesis from erythrocytes during blood storage. Front Physiol 11: 712, 2020.
112. Shao H, Im H, Castro CM, Breakefield X, Weissleder R and Lee H: New technologies for analysis of extracellular vesicles. Chem Rev 118: 1917-1950, 2018.
113. Nguyen DB, Ly TB, Wesseling MC, Hittinger M, Torge A, Devitt A, Perrie Y and Bernhardt I: Characterization of microvesicles released from human red blood cells. Cell Physiol Biochem 38: 1085-1099, 2016.
114. Noubouossie DF, Henderson MW, Mooberry M, Ilich A, Ellsworth P, Piegore M, Skinner SC, Pawlinski R, Welsby I, Renné T, *et al*: Red blood cell microvesicles activate the contact system, leading to factor IX activation via 2 independent pathways. Blood 135: 755-765, 2020.
115. van der Pol E, Coumans F, Varga Z, Krumrey M and Nieuwland R: Innovation in detection of microparticles and exosomes. J Thromb Haemost 11 (Suppl 1): S36-S45, 2013.
116. Lawrie AS, Albanyan A, Cardigan RA, Mackie IJ and Harrison P: Microparticle sizing by dynamic light scattering in fresh-frozen plasma. Vox Sang 96: 206-212, 2009.
117. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, *et al*: Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7: 1535750, 2018.

118. Muszynski JA, Bale J, Nateri J, Nicol K, Wang Y, Wright V, Marsh CB, Gavrilin MA, Sarkar A, Wewers MD and Hall MW: Supernatants from stored red blood cell (RBC) units, but not RBC-derived microvesicles, suppress monocyte function in vitro. Transfusion 55: 1937-1945, 2015.
119. Turpin D, Truchetet ME, Faustin B, Augusto JF, Contin-Bordes C, Brisson A, Blanco P and Duffau P: Role of extracellular vesicles in autoimmune diseases. Autoimmun Rev 15: 174-183, 2016.
120. Vasconcelos MH, Caires HR, Ābols A, Xavier CPR and Linē A: Extracellular vesicles as a novel source of biomarkers in liquid biopsies for monitoring cancer progression and drug resistance. Drug Resist Updat 47: 100647, 2019.
121. Naito Y, Yoshioka Y, Yamamoto Y and Ochiya T: How cancer cells dictate their microenvironment: Present roles of extracellular vesicles. Cell Mol Life Sci 74: 697-713, 2017.
122. Zecher D, Cumpelik A and Schifferli JA: Erythrocyte-derived microvesicles amplify systemic inflammation by thrombin-dependent activation of complement. Arterioscler Thromb Vasc Biol 34: 313-320, 2014.
123. Liu C, Zhao W, Christ GJ, Gladwin MT and Kim-Shapiro DB: Nitric oxide scavenging by red cell microparticles. Free Radic Biol Med 65: 1164-1173, 2013.
124. Kim-Shapiro DB, Lee J and Gladwin MT: Storage lesion: Role of red blood cell breakdown. Transfusion 51: 844-851, 2011.
125. Said AS and Doctor A: Influence of red blood cell-derived microparticles upon vasoregulation. Blood Transfus 15: 522-534, 2017.
126. Kamm A, Przychodzen P, Kuban-Jankowska A, Jacewicz D, Dabrowska AM, Nussberger S, Wozniak M and Gorska-Ponikowska M: Nitric oxide and its derivatives in the cancer battlefield. Nitric Oxide 93: 102-114, 2019.
127. Oliveira GP Jr, Zigon E, Rogers G, Davodian D, Lu S, Jovanovic-Talisman T, Jones J, Tigges J, Tyagi S and Ghiran IC: Detection of extracellular vesicle RNA using molecular beacons. iScience 23: 100782, 2020.
128. Miyashita Y, Ishikawa K, Fukushima Y, Kouwaki T, Nakamura K and Oshiumi H: Immune-regulatory microRNA expression levels within circulating extracellular vesicles correspond with the appearance of local symptoms after seasonal flu vaccination. PLoS One 14: e0219510, 2019.
129. Okamoto M, Fukushima Y, Kouwaki T, Daito T, Kohara M, Kida H and Oshiumi H: MicroRNA-451a in extracellular, blood-resident vesicles attenuates macrophage and dendritic cell responses to influenza whole-virus vaccine. J Biol Chem 293: 18585-18600, 2018.
130. Liu Z, Miao T, Feng T, Jiang Z, Li M, Zhou L and Li H: miR-451a inhibited cell proliferation and enhanced tamoxifen sensitive in breast cancer via macrophage migration inhibitory factor. Biomed Res Int 2015: 207684, 2015.
131. Yamada Y, Arai T, Sugawara S, Okato A, Kato M, Kojima S, Yamazaki K, Naya Y, Ichikawa T and Seki N: Impact of novel oncogenic pathways regulated by antitumor miR-451a in renal cell carcinoma. Cancer Sci 109: 1239-1253, 2018.
132. Nobre CC, de Araújo JM, Fernandes TA, Cobucci RN, Lanza DC, Andrade VS and Fernandes JV: Macrophage migration inhibitory factor (MIF): Biological activities and relation with cancer. Pathol Oncol Res 23: 235-244, 2017.
133. Almizraq RJ, Kipkeu BJ and Acker JP: Platelet vesicles are potent inflammatory mediators in red blood cell products and washing reduces the inflammatory phenotype. Transfusion 60: 378-390, 2020.
134. Nelson KA, Aldea GS, Warner P, Latchman Y, Gunasekera D, Tamir A, Gernsheimer T, Bolgiano D and Slichter SJ: Transfusion-related immunomodulation: Gamma irradiation alters the effects of leukoreduction on alloimmunization. Transfusion 59: 3396-3404, 2019.
135. Sanchez R, Lee TH, Wen L, Montalvo L, Schechterly C, Colvin C, Alter HJ, Luban NL and Busch MP: Absence of transfusion-associated microchimerism in pediatric and adult recipients of leukoreduced and gamma-irradiated blood components. Transfusion 52: 936-945, 2012.
136. Bohlius J, Bohlke K, Castelli R, Djulbegovic B, Lustberg MB, Martino M, Mountzios G, Peswani N, Porter L, Tanaka TN, *et al*: Management of cancer-associated anemia with erythropoiesis-stimulating agents: ASCO/ASH clinical practice guideline update. Blood Adv 3: 1197-1210, 2019.