

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

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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.

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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 Adsol-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

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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 $\leq 7-8$ 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 <i>et al</i> , 2012	Blood transfusion and cancer surgery outcomes: A continued reason for concern.	(10)
Deeb <i>et al</i> , 2020	allogeneic leukocyte-reduced red blood cell transfusion is associated with postoperative infectious complications and cancer recurrence after colon cancer resection.	(28)
Tamini <i>et al</i> , 2021	Colon Cancer Surgery: Does preoperative blood transfusion influence short-term postoperative outcomes?	(29)
Qiu <i>et al</i> , 2016	Impact of perioperative blood transfusion on immune function and prognosis in colorectal cancer patients.	(30)
Acheson <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 2012	Does blood transfusion increase the chance of recurrence in patients undergoing surgery for lung cancer?	(34)
Seon <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 2013	Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions.	(37)
B, No impact		
Author (year)	Title	(Refs.)
Baguena <i>et al</i> , 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 <i>et al</i> , 2013	Blood transfusion does not adversely affect survival after elective colon cancer resection: A propensity score analysis.	(43)
Hunsicker <i>et al</i> , 2019	Transfusion of red blood cells does not impact progression-free and overall survival after surgery for ovarian cancer.	(44)
Zaw <i>et al</i> , 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-1 β , 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

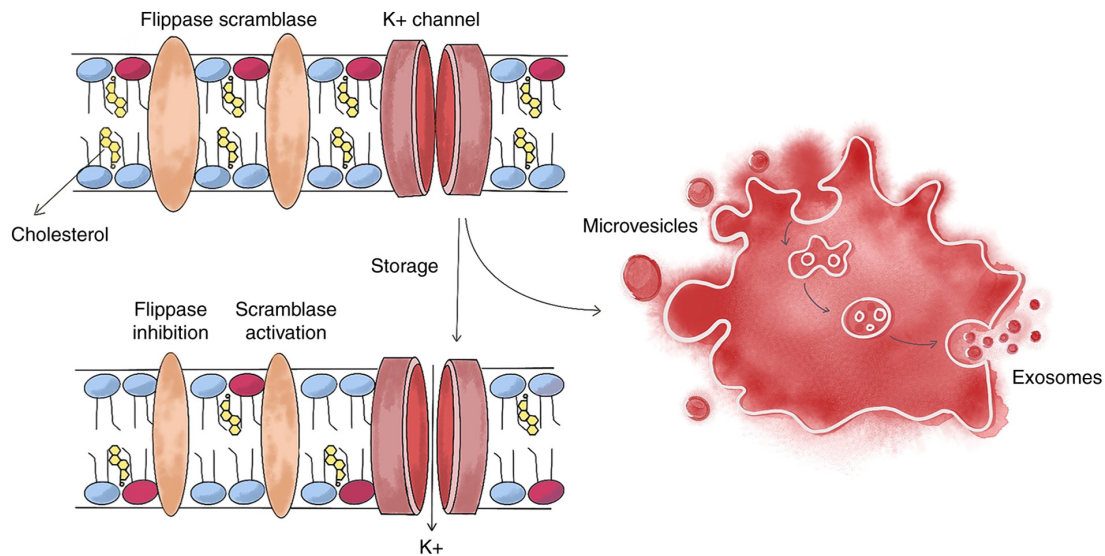


Figure 1. Lipid composition of RBCs. RBCs undergo storage lesions during storage, whereby cell membranes are damaged and extracellular vesicles are released. RBCs, red blood cells.

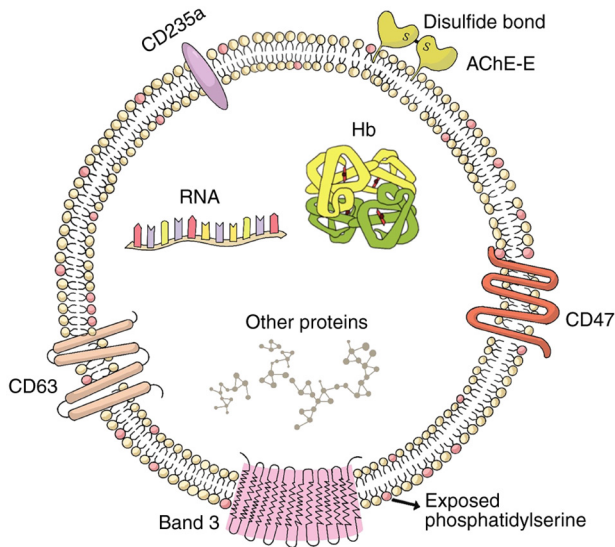


Figure 2. Biomarkers and contents contained within RBC-derived extracellular vesicles. Hb, hemoglobin; AChE-E, acetylcholinesterase-erythrocytes.

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 Ca²⁺ is added, exposure of PS on the surface of RBCs and the formation of EVs are associated with increased Ca²⁺ levels inside the cell (102,103,110). Although citrates in the RBC preservation solution may chelate Ca²⁺ to a reduce Ca²⁺ in the plasma, it has been reported that EDTA, heparin and citrates are unable to completely chelate Ca²⁺ in extracellular medium, such that Ca²⁺ 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/Ca²⁺ 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 Ca²⁺ levels (106). Therefore, the association between changes in the intracellular Ca²⁺ 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 IEVs (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

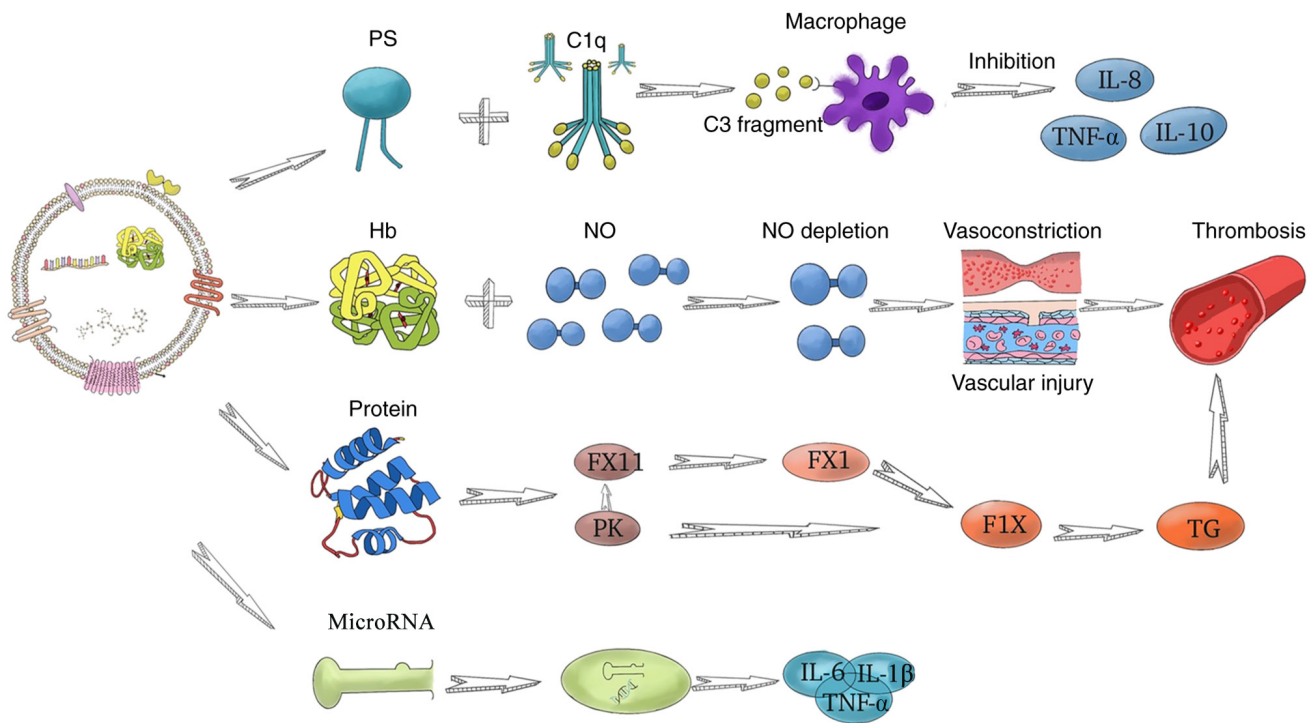


Figure 3. Mechanism of stored RBC-derived EVs in TRIM in patients with cancer receiving transfusions. PS on the surface of RBC-derived EVs are able to bind to C1q in the blood to activate the classical pathway of complement and inhibit immune cells from releasing a variety of immune factors, such as 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 5×10^6 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 ($\text{Hb} \leq 7\text{-}8 \text{ g/l}$).

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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.

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