Published in final edited form as:
Transfusion. 2011 April ; 51(4): 886–893. doi:10.1111/j.1537-2995.2011.03099.x.

Microparticles in Stored RBC as Potential Mediators of Transfusion Complications

Wenche Jy, PhD[1], **Marco Ricci, MD**[2], **Sherry Shariatmadar, MD**[3], **Orlando Gomez-Marin, PhD**[4], **Lawrence H Horstman**[1], and **Yeon S Ahn, MD**[1]

[1] Wallace H Coulter Platelet Laboratory, Division of Hematology & Oncology, Department of Medicine, University of Miami School of Medicine

[2] Department of Surgery, University of Miami School of Medicine

[3] Department of Pathology, University of Miami School of Medicine

[4] Department of Public Health & Epidemiology, University of Miami School of Medicine

Abstract

This article reviews evidence for the involvement of cell-derived microparticles (MP) in transfusion-related adverse events. The controversy concerning possible added risk of older vs. fresher stored blood is also reviewed, and is consistent with the hypothesis that MP are involved with adverse events. Although all types of circulating MP are discussed, the emphasis is on red cell-derived MP (RMP). The evidence is particularly strong for involvement of RMP in transfusion-related acute lung injury (TRALI), but also for post-operative thrombosis. However, this evidence is largely circumstantial. Work in progress to directly test the hypothesis is also briefly reviewed.

Introduction

Red blood cells (RBC) stored in the blood bank undergo a series of physical and chemical changes and release many potentially hazardous products, increasing with time, resulting in the so-called “storage lesion”[1]. In view of some but not all recent studies, it is widely believed that transfusion with younger blood carries less risk of adverse reactions than older blood[2–6]. However, there is little agreement on the “safe” age of blood, nor is it clearly understood why older blood may carry increased risks.

It has been known since the 1970’s that stored whole blood, platelet concentrates, and RBC release submicron-sized fragments of the cells’ plasma membranes to the supernatant, and that their numbers increase with time of storage [7, 8]. These fragments, or *vesicles*, are commonly termed “microparticles” (MP) and constitute one aspect of the storage lesion. A series of studies has shown that MP released from blood cells exhibited strong procoagulant and proinflammatory activities[9–12]. In addition, red cell microparticles (RMP) also contain hemoglobin, a potent scavenger of nitric oxide (NO), which has been shown to modulate vascular contractility *via* NO pathway[13]. Several relevant reviews have appeared in recent years[9–12]. This review will focus on the generation of MP during blood storage, and on evidence supporting the hypothesis that MP act as a mediator of transfusion-related inflammatory and thrombotic complications.

Corresponding Author: Wenche Jy, PhD, 1600 NW 10th Ave, R-36A, Miami, FL 33176, Telephone: 305-243-6617, Fax: 305-243-5957, wjy@med.miami.edu.

Conflict of Interest: None

Risk Associated with Age of RBC

It is our working hypothesis that RMP or other MP released from stored RBC may contribute to at least some of the adverse effects of transfusion that occasionally occur. Since RMP are released steadily with time and accumulate, it is expected that associated adverse effects would also increase with time. In this section, we first briefly review reports of increased risk of older blood, then more recent reports doubting those findings, and finally draw some tentative conclusions.

In 2006, the first of a series of reports appeared which challenged the safety of blood aged up to the allowed 42 days. These reports, chiefly by Koch and colleagues, presented evidence of significantly increased mortality and adverse events associated with blood aged >14 days, and suggested that such risks increase continuously with time of storage[2–4].

More recently, major questions about the validity of the findings of Koch et al have appeared. It was pointed out that patients in the study of Koch et al who received older blood (>14 days) also most frequently received 6 or more units, which is known to be independently associated with increased mortality[14]. After adjustment for this and other confounders, the significance of the findings of Koch et al almost disappeared[14].

Edgren et al[5], in the largest and most recent study yet published, analyzed data on 404,959 transfusions between 1995–2002. Contrary to other reports, they found no significant relation at all between age of blood and 7-day mortality. A “tendency” (5% increase in mortality rate) was noted for blood aged 30–42 days. They conclude that any excess mortality associated with blood age is <5%, which they point out is much less than found in previous smaller studies, suggesting that confounding factors have distorted results in previous studies.

Also in 2010, Van Stratten et al[15] analyzed 9 years of transfusion data for coronary bypass at a large hospital, classified in 3 groups, (A) n=1422 who received blood age 0–14 days, (B) n=1719 received blood stored >14 days, (C) n=2175 received mixed but at least 1U >14 days. All were followed for average 170 days. They found no significant association of age of RBC transfused and mortality, either early or later. Similarly, Robinson et al[16] analyzed data on 32,580 patients who underwent percutaneous coronary intervention (PCI), of whom 909 received blood, of mean age 25 +/− 10 days. They then divided the 909 into those who received blood <25 days old (n = 352) *vs.* >25 days (n = 360). They detected no significant difference in 30-day mortality between these groups. In other findings, they confirmed that mortality was associated with volume of blood transfused; and that any transfusion is associated with higher mortality compared to no transfusion. Elkelboom et al[6] analyzed data on about 7,000 patients receiving a total of 21,400 units of blood of median age 17 days. They divided recipients into quartiles by blood age (0–13, 14–17, 18–22, 23–42 days) and detected “a modest independent association” between duration of storage of RBC and in-hospital mortality.

In view of these large retrospective studies, it now appears that the reports of Koch et al and related studies greatly exaggerated the risk associated with older blood, at least with respect to mortality statistics. Adverse events (other than mortality) are more difficult quantitatively evaluate by retrospective analysis. This issue needs to be addressed by prospective controlled studies with well-defined patient groups. For example, Gauvin et al[17] showed that stable critically ill children who receive RBC units with storage times longer than 2 to 3 weeks may be at greater risk of developing new or progressive multiple organ dysfunction syndrome (MODS) in a prospective randomized controlled trial.

Risk of older blood limited to specific circumstances?

Although the recent reports listed above found little added risk of older blood, reports of increased risk of older blood continue to appear. If the more recent reports of adverse effects of older blood are free of confounding factors, then it is possible that specific patient groups, such as those with traumatic bleeding requiring massive transfusions[18], or critically ill children[19], are more sensitive to adverse reactions to factors in aged blood. The US Army has reported significant problems with blood of age >30 days[20]. Tsai et al found that adverse effects of time of blood storage was limited to the microvasculature, and would not necessarily be evident at the systemic level[21].

It is clear that the hazards of aged blood were overstated by Koch et al[4] but some recent prospective controlled studies suggest that at least some degree of risk increases with time. The extent of this risk may depend on particular patient populations or the type of situation requiring transfusion, e.g. trauma.

MP generation during blood storage

Studies of RMP in blood bank products

In the late 1970's and early 1980's, a number of studies of MP released from blood stored for transfusion appeared, particularly on MP from RBC, here termed RMP. It was reported that the release of RMP from stored blood over time was related to the shape transition from discoid to echinocytic to spherocytic, supported by scanning EM micrographs; and both paralleled sensitivity of the RBC to phospholipase C-induced shape change[7, 8]. The rate of these changes was almost zero for the first 10 days, followed by a steep rise from days 10 to 15, then little change until a further steady rise at 5–8 weeks. These effects were largely blocked or reversed up to 30 days by a "rejuvenating" additive consisting of glucose, pyruvate, inosine, adenine, and phosphate. In addition, RMP are known to exist in two size classes, which differ in physical and chemical properties[22, 23]. The "large" ones (MP, 0.1–1.1 um) are those normally measured in clinical studies while the "small" ones (nanoparticles, 60–80 nm) are more difficult to detect in flow cytometry and to isolate by centrifuge. Both size classes are produced by erythrocytes. The compositional difference of these two types of particles will be discussed in a later section.

Multiple species of MP in stored PC

The presence of other MP species such as platelet MP (PMP) or leukocyte MP (LMP) in stored RBC has been mostly ignored. A recent study showed that multiple species of MP were generated in stored non-leukoreduced PC[24]. Although RMP are the predominant species, significant amount of PMP and LMP are also generated during blood storage. The time-course of generation of the MP subtypes varied considerably. For RMP, there was little increase up to day 10, but thereafter rose steadily with time even after day 42. For PMP, counts rose steadily from day 0 and peaked at day 20. For LMP, there was no significant change in the first 20 days but continuous increases after day 30. The degree of neutrophil activation correlated well with PMP levels and the thrombin generation correlated with PMP and RMP. These data suggest increasing proinflammatory and procoagulant potential with time of storage.

However, Sugawara et al[25] showed that pre-storage leukofiltration of whole blood significantly decreased post-storage PMP count. We have confirmed that finding, and in addition, found that leukoreduction also substantially reduced RMP generation (unpublished). Thus, leukoreduction not only removes immunogenic leukocytes but also reduces MP generation during storage. This effect may contribute additional benefits of leukoreduction for reducing risks of transfusion.

Composition of RMP

Cole et al demonstrated that for most of blood groups (A, B, H, P_1), the antigens are steadily released for up to 6–8 weeks in association with membrane vesicles (MP), since blood group reactivity could be sedimented at 100,000 ×g[26]. Kriebardis et al performed a detailed analysis of the components of RMP over time in stored PC and compared them with ghosts of whole RBC[27]. That study included electron microscopy (EM) of colloidal gold stained RMP, which were 0.1–0.2 um. RMP constituents testing positive by blotting were stomatin, synexin, flotillins 1 and 2, sorcin, band 3, aquaporin, CD47, caspase and procaspase 3 and 8, Fas, FADD and abundant IgG. Nearly all increased steadily with time of storage, while declining in the whole RBC ghosts. Several were lipid raft proteins, and the proteins involved with apoptosis tend to confirm that RBC undergo a kind of apoptosis. There was notable evidence of oxidized products increasing to 5-fold in proportion to that of the RBC ghosts by day 15. They concluded that RMP release functions mainly to dispose of harmful agents from senescent cells. Earlier study by Muller and Lutz demonstrated preferential binding of autologous IgG to RMP[28]. The view that RMP function mainly to dispose of damaged or harmful agents has been suggested by others, recently by Willekens et al[29], but it is unlikely that is the only or main function of MP shedding.

Salzer et al[30] studied RMP from stored blood at intervals to 50 days and compared their properties to the corresponding RBC, and to RMP produced *in vitro* by calcium ionophore. RMP from stored blood were similar to those made *in vitro*, with notable exception of some proteins. Their work is novel in employing atomic force microscopy (AFM) to obtain size histograms of RMP, which ranged from 50–200 nm; but conditions of these measurements may alter the particles compared to flow cytometry. They also measured thrombin generation and phosphotidylserine (PS) exposure. They performed density fractionations in which they assessed by blotting acetylcholinesterase (AChE), band 3, stomatin, CD55 (a.k.a. DAF), flotillin-2 and Duffy antigens. They emphasized new insights on the process of storage-induced RBC vesiculation, i.e.. that it is “raft-based” and “stomatin-specific”.

Bosman et al[31] performed a semi-quantitative proteomic analysis of RMP and the parent RBC membranes. Although a large number of identified peptide fragments could not be assigned to individual proteins (data online lists about 20,000 fragments), the authors identified a total of 257 different proteins. For example, semaphorin 7A and peroxiredoxins decreased in the parent RBC with age of storage but simultaneously increased in the microparticles (RMP) and nanoparticles (RNP). Many complement components were identified on the RMP, including fragments of C1q, C1r, C1s, C3, C4 and C9. Of interest, a comparison of RMP and RNP showed, unexpectedly, that RNP were 100-fold enriched in complement proteins compared to the RBC membrane, and 10-fold enriched compared to RMP. Immunoglobulins were also greatly enriched in the RNP. When taken together with the proteomic study of whole RBC (membrane and cytosolic components) by Pasini et al[32], this study is highly suggestive that RMP have proinflammatory potential owing to the presence of complement and immunoglobulins. Several proteins involved in coagulation such as phospholipid scramblase 1, plasminogen precursor, fibrinogen beta chain precursor, and beta-2-glycoprotein 1 were also detected on RMP.

MP-mediated proinflammatory activities

RMP as mediators of the complement (C) system

The complement (C) system is a major mediator of inflammation *via* many pathways. Erythrocytes have complement receptors and play vital roles in innate immunity and inflammation *via* the C system. For example, dysregulation of C in relation to RBC was recently linked to venous insufficiency of the lower extremeties, involving CD35 (CR1)[33].

A broad consensus indicates that C is the underlying cause of many adverse effects of blood transfusion including hemolytic reaction[34–36], anaphylactic reaction[37, 38], and TRALI[39].

It has been shown that C receptor 1 (CR1, a.k.a. CD35) is enriched on RMP from ATP-depleted RBC, which was proposed to explain the progressive loss of CR1 on RBC with storage[40]. CR1 is a central player in RBC immune function, chiefly by elimination of immune complex[41, 42]. The C5b-9 membrane attack complex (MAC) is selectively shed on MP from platelets[43], endothelial cells[44] and other cells as a defensive mechanism, and is probably true also for RBC[45]. Several authors have proposed that a major function of MP shedding is to dispose of harmful agents from cells, such as are likely to be opsonized by C[29, 46]. The previously mentioned proteomic studies of RMP showed great enrichment in complement and IgG[31]. These observations suggest that RMP in stored blood may be involved in some types of C-mediated adverse effects of transfusion.

Interactions of MP with leukocytes

MP from platelets (PMP) and from endothelial cells (EMP) were shown to adhere to and activate leukocytes [47, 48], and many authors have since confirmed and extended those findings. Comparatively little research has been done on possibly similar interactions of RMP, probably because RMP do not possess selectins (C62P, CD62E, CD62L) and therefore cannot interact *via* those adhesins. However, other mechanisms for interaction can occur. Gasser and Schifferli[49] demonstrated that leukocyte MP (LMP) bind to intact RBC in a C-dependent fashion; i.e., binding of C3 followed by activation of the classical pathway on LMP yielded C3 fragments, resulting in capture by RBC *via* CR1 (similar to capture of immune complex). This binding may well act in reverse, i.e., C fixation occurring on RMP followed by binding to leukocytes. In addition, expression of PS on any MP can lead to binding to leukocytes since they have a specific PS receptor[50].

MP as a potential mediator for TRALI

Transfusion-related acute lung injury (TRALI) is among the most serious of transfusion-related adverse events with high morbidity and mortality. The underlying mechanism has been unclear, but recent work persuasively demonstrates a 2-hit scenario. Using a sheep model, Tung et al have shown that if the animal is first sensitized by inducing inflammation *via* lipopolysaccharide (LPS), which primes neutrophils, then administration of either the supernatant of 5-day aged platelets or the supernatant of 42-day aged RBC results in neutrophil sequestration in the lungs and full-blown TRALI in 80–90% of animals[51, 52]. These findings are consistent with current consensus that excessive neutrophil activation plays a pivotal role in the pathogenesis of TRALI[53]. Although the identity of the substance in the supernatants responsible for this outcome has not been established, we postulate that PMP and/or RMP are likely to be the main mediators for TRALI, by the following evidence. First, it has been shown that PMP can bind and activate neutrophils *via* P-selectin – PSGL-1 interaction [47, 54]. Second, complement and IgG are enriched in RMP from storage lesion[31]. The RMP-bound IgG and complement can activate neutrophils *via* Fc receptors of neutrophils. Third, it has been reported that CD40L released from stored platelet concentrates is also a potential mediator for TRALI[55]. Studies have demonstrated that the majority of CD40L in blood is actually MP-bound[56–58]. Our group showed that much of CD40L could be removed by 0.1 um filtration[57].

MP-mediated thrombotic complications

Because microparticles have been implicated in thrombosis by work from our lab[59–61] and others[62–65], and exhibit potent procoagulant activity[59, 62], they may contribute significantly to post-transfusion thrombosis. The most important procoagulant property of MP is their

expression of the anionic phospholipid, phosphatidyl serine (PS), which serves for assembly of the coagulation factors into active complexes for thrombin generation. We developed an assay for MP-mediated thrombin generation[66], and compared *in vitro* the relative profiles of PMP, EMP and RMP[67]. Marked differences were observed. EMP exhibited short lag time (2–3 min) but low thrombin peak amplitude. In contrast, RMP show a large thrombin peak but very long lag time (>15 min). PMP exhibited intermediate lag time (8–10 min) and highest thrombin peak. The lag time is inversely correlated with tissue factor (TF) expression, and thrombin peak seems to be proportional to PS expression.

The thrombogenic potential of PMP is well-recognized but some evidence suggests also involvement of RMP. Binder's group measured formation of thrombin-antithrombin (TAT) to measure prothrombotic activity in mice, and observed that RMP injected in normal mice had no effect on TAT, but if injected into mice fed a high-fat diet, TAT increased markedly[68]. This observation is consistent with findings concerning TRALI, in the sense that MP-mediated adverse effects of transfusion may occur only in specific patient groups, i.e., pre-existing inflammation for TRALI, high cholesterol for thrombosis due to RMP. Therefore, the greater risk of aged blood may apply only to sensitive or sicker patient groups.

Since we have observed significant amounts of PMP in packed cells (PC), but not in leuko-reduced PC (unpublished), we postulate that PMP may work synergistically with RMP in mediating some transfusion-related thrombotic complications. In a recent study, Spinella et al reported a higher incidence (34%) of DVT in trauma patients receiving older blood (28 days or more) compared to blood <28 days old (16%); $p<0.02$[18]. This observation is consistent with the hypothesis that cell-derived MP may contribute to the DVT since it is known that MP increase with time of storage. They also observed increased in hospital mortality with the older blood (16% *vs.* 7% for younger blood).

Post-operative cognitive impairment (POCI) is a well recognized complication[69–71]. To our knowledge, it is not established if this complication is exacerbated by transfusion, but there is evidence of MP involvement in ischemic brain disease[72]. We demonstrated correlation between PMP levels and ischemic brain disease [73–75]. The frequency of POCI was reduced in off-pump *vs.* on-pump CABG procedures[76], which is also consistent with possible involvement of MP, as it is known that exposure of platelets to artificial surfaces induces their activation and PMP release[77]. However, the hypothesis that blood transfusions contribute to post-surgical thrombosis requires direct testing.

Testing the Hypothesis of MP Involvement in Transfusion Complications

The work reviewed suggests but does not prove that cell-derived MP may contribute to transfusion-related complications. In view of concerns about older blood, our laboratory will more directly test this hypothesis. The main strategy for the *in vivo* component of the study is to compare clinical outcomes and proinflammatory/procoagulant biomarkers in CABG patients who received either normal PC (n = 100–150 target) or washed PC (n = 100–150 target), using the rationale that washing will remove the accumulated MP from the storage lesion. A second component of the study is to investigate *in vitro* the rate of MP production and the MP properties in stored blood over time, and their composition in proteomic analysis, by methods designed to improve on previous studies.

A major challenge, which was anticipated, is the fact that significant transfusion complications are very rare. Our patient population is unlikely to be large enough to detect significant differences in adverse events between these two groups. To address this short coming, we are looking at laboratory biomarkers that could suggest more serious adverse effects in some patients. These are markers of inflammation, oxidation, procoagulant

activity, and others. Even if it is not possible to draw clear conclusions about the role of MP in adverse effects of transfusion, a wealth of additional data is being compiled that is expected to elucidate important aspects of transfusion medicine.

Acknowledgments

This work is supported by grants from Wallace H Coulter Foundation and a grant from National Institute of Health (1R01HL098031).

References

1. Hess JR. Red cekk storage. J Proteomics. 2010; 73(3):368–373. [PubMed: 19914410]
2. Koch CG, Li L, Duncan AZ, et al. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery grafting. Crit Care Med. 2006; 34(6):1608–1616. [PubMed: 16607235]
3. Koch CG, Khandwala F, Li L, et al. Persistent effect of red cell transfusion on health related quality of life after cardiac surgery. Ann Thorac Surg. 2006; 82(1):13–20. [PubMed: 16798179]
4. Koch CG, Li L, Sessler DZ, et al. Duration of red cell storage and complications after cardiac surgery. New Engl J Med. 2008; 358(12):1229–1239. [PubMed: 18354101]
5. Edgren G, et al. Duration of red blood cell storage and survival of transfused patients. Transfusion. 2010; 50(6):1185–1195. [PubMed: 20158690]
6. Elkelboom JW, et al. Duration of red cell storage before transfusion and in-hospital mortality. Am Heart J. 2010; 159(5):737–743. [PubMed: 20435180]
7. Shukla SD, Coleman R, Finean JB, Michell RH. The use of phospholipase c to detect structural changes in the membranes of human erythrocytes aged by storage. Biochim Biophys Acta. 1978; 512(2):341–349. [PubMed: 213113]
8. Rumsby MG, Trotter J, Allan D, Michell RH. Recovery of membrane micro-vesicles from human erythrocytes stored for transfusion: a mechanism for the erythrocyte discocyte-to-sphere shape transformation. Biochem Soc Trans. 1977; 5(1):126–128. [PubMed: 892138]
9. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. Thromb Haemost. 2009; 101(3):439–451. [PubMed: 19277403]
10. Horstman LL, Jy W, Bidot C, Nordberg ML, Minagar A, Alexander JS, et al. Potential roles of cell-derived microparticls in ischemic brain disease. Neurol Res. 2009; 31(8):799–806. [PubMed: 19723448]
11. Rubin O, Crettaz D, Tissot JD, Lion N. Microparticles in stored red blood cells: submicron clotting bombs? Blood Transfus. 2010; 8(supl3):S 31–38.
12. Leroyer AS, Anfosso F, Lacroix R, Sabatier F, Simoncini S, Njock SM, et al. Endothelial-derived microparticles: Biological conveyors at the crossroad of inflammation, thrombosis and angiogenesis. Thromb Haemost. 2010; 104(3):456–463. [PubMed: 20664896]
13. Gladwin MT, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. Curr Opin Hematol. 2009; 16(6):515–23. [PubMed: 19701085]
14. Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. Vox Sang. 2009; 96(2):93–103. [PubMed: 19152602]
15. vanStratten AHM, et al. Effect of duration of red blood cell storage on early and late mortality after coronary artery bypass grafting. J Thorac Cadiovasc Surg. 2010 Jul 9. epub preprint.
16. Robinson SD, et al. Red blood cell storage duration and mortality in patients undergoing percutaneous coronary intervention. Am Heart J. 2010; 159(5):876–881. [PubMed: 20435199]
17. Gauvin, f; Spinella, pc; Lacroix, j; Choker, g; Ducruet, t; Karam, o, et al. Association between length of storage of transfused red blood cells and multiple organ dysfunction syndrome in pediatric intensive care patients. Transfusion. 2010; 50(9):1902–1912. [PubMed: 20456697]
18. Spinella PC, Carroll CL, Staff I, Gross R, McQuay J, Keibel L, et al. Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries. Crit Care. 2009; 13(5):R 151.

19. Karam O, Tucci M, Bateman ST, Ducruet T, Spinella PC, Randolph AG, et al. Association between length of storage of red blood cell units and outcome of critically ill children: a prospective observational study. Crit Care. 2010; 14(2):R 57.
20. Beekley AC, Starns BW, Sebesta JA. Lessons learned from modern military surgery. Surg Clin N Am. 2007; 87:157–184. [PubMed: 17127127]
21. Tsai AG, Cabrales P, Intaglietta M. Microvascular perfusion upon exchange transfusion with stored red blood cells in normovolemic conditions. Transfusion. 2004; 44:1626–1634. [PubMed: 15504169]
22. Allan D, Thomas P, Limbrick AR. The isolation and characterization of 60nm vesicles ("nanovesicles") produced during ionophore A23187-induced budding of human erythrocytes. Biochem J. 1980; 188:881–887. [PubMed: 6781476]
23. Jy W, Horstman LL, Ahn YS. Microparticle size and its relation to composition, functional activity, and clinical significance. Semin Thromb Hemost. 2010; 36(8):876–880. [PubMed: 21049388]
24. Jy W, Bidot C Jr, Johansen ME, Horstman L, Shariatmadar S, Ricci M, et al. Red-Cell Microparticles Released From Stored Packed Cells: Possible Contributing Factor to Adverse Responses to Transfusion [Presented, 52nd American Society of Hematology Annual Meeting, December 4–7; Orlando, FL]. Blood. 2010; 116(21):154. Ab#342. [PubMed: 20634384]
25. Sugawara A, Nollet KE, Yajima K, Saito S, Ohto H. Preventing platelet-derived microparticle formation--and possible side effects-with prestorage leukofiltration of whole blood. Arch Pathol Lab Med. 2010; 134(5):771–775. [PubMed: 20441510]
26. Cole WF, Rumsby MG, Longster GH, Tovey LA. The release of erythrocyte membrane antigens to the plasma as membrane microparticles during the storage of blood for transfusion. Biochem Soc Trans. 1978; 6(6):1375–1378. [PubMed: 744432]
27. Kriebardis AC, Antonelou MH, Stamoulis KE, et al. RBC-derived vesicles during storage: ultrastructure, protein composition, oxidation and signalling components. Transfusion. 2008; 48(9):1943–1953. [PubMed: 18564399]
28. Muller H, Lutz HU. Binding of autologous IgG to human red blood cells before and after ATP-depletionL Selective exposure of binding sites (autoantigens) on spectrin-free vesicles. Biochim Biophys Acta. 1983; 729(2):249–257. [PubMed: 6830791]
29. Willekens FL, Werre JM, Roerdinkholder YA, et al. Erythrocyte vesiculation: a self-protective mechanism? Br J Haematol. 2008; 141(4):549–556. [PubMed: 18419623]
30. Salzer U, Zhu R, Luten M, Isobe H, et al. Vesicles generated during storage of red cells are rich in the lipid raft marker stomatin. Transfusion. 2008; 48:451–462. [PubMed: 18067507]
31. Bosman GJ, Lasonder E, Luten M, et al. The proteome of red cell membranes and vesicles during storage in blood bank conditions [for detailed lists see supplementary materials online as cited]. Transfusion. 2008; 48:827–835. [PubMed: 18346024]
32. Pasini EM, Kirkegaard M, Mortensen P, et al. In-depth analysis of the membrane and cytosolic proteome of red blood cells [with editorial p779]. Blood Cells Mol Dis. 2006; 108(3):791–801.
33. Zhang L, Zhang BG, Zhang JW, Zhang H. Immune function of erythrocytes in patients with chronic venous insufficiency of the lower extremeties. Chin Med J. 2007; 120(24):2224–2228. [PubMed: 18167207]
34. Salama A, Mueller-Eckhardt C. Delayed hemolytic transfusion reaction [DHTR]. Evidence for complement activation involving allogeneic and autologous red cells. Transfusion. 1984; 24(3): 188–193. [PubMed: 6610232]
35. Davenport RD. Pathophysiology of hemolytic transfusion reaction. Semin Hematol. 2005; 42(3): 165–168. [PubMed: 16041666]
36. Yazdanbakhsh K. Controlling the complement system for prevention of red cell destruction. Curr Opin Hematol. 2005; 12(2):117–122. [PubMed: 15725901]
37. Teisner B, Brandslund J, Grunnet N, et al. Acute complement activation during an anaphylactoid reaction to blood transfusion and the disappearance rate of C3c and C3d from the circulation. J Clin Lab Immunol. 1983; 12(2):63–67. [PubMed: 6606048]
38. Gilstad CW. Anaphylactic transfusion reactions. Curr Opin Hematol. 2003; 10(6):419–423. [PubMed: 14564171]

39. Fabron A Jr, Lopez LB, Bordin JO. Transfusion-related acute lung injury [Review)]. J Bas Pneumol. 2007; 33(2):206–210.
40. Pascual M, Lutz HU, Steiger G, Stammler P, Schifferli JA. Release of vesicles enriched in complement receptor 1 from human erythrocytes. J Immunol. 1993; 151(1):397–404. [PubMed: 8326133]
41. Gibson NG, Waxman FJ. Relationship between immune complex binding and release and quantitative expression of the complement receptor, Type 1 (CR1, CD35) on human erythrocytes. Clin Immunol Immunopath. 1994; 70(2):104–113.
42. Pascual M, Schifferli JA. Another function of erythrocytes: transport of circulating immune complexes. Infusionsther Transfusionsmed. 1995; 22(5):310–315. [PubMed: 8924746]
43. Sims PJ, Wiedmer T. The response of human platelets to activated components of the complement system. Immunol Today. 1991; 12(9):338–341. [PubMed: 1755945]
44. Hamilton KK, Hattori R, Esmon CT, Sims PJ. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem. 1990; 265(7):3809–3814. [PubMed: 2105954]
45. Pilzer D, Gasser O, Moskovich O, Schifferli JA, Fishelson Z. Emission of membrane vesicles: roles in complement resistence, immunity and cancer. Springer Semin Immunopath. 2005; 27(3): 375–387.
46. Kriebardis AC, Antonelou MH, Stamoulis KE, et al. Storage-dependent remodeling of the red blood cell membrane is associated with increased immunoglobulin G binding, lipid raft rearrangement, and caspase activation. Transfusion. 2007; 47:1212–1220. [PubMed: 17581156]
47. Jy W, Mao WW, Horstman LL, Tao J, Ahn YS. Platelet microparticles bind, activate and aggregate neutrophils in vitro [with color photomicrographs]. BCMD (Blood Cells, Molecules and Diseases). 1995; 21(3):217–231.
48. Barry OP, Pratico D, FitzGerald G. Platelet microparticles enhance adhesive interactions between monocytes and endothelial cells. J Clin Invest. 1997; 45(3):271A.
49. Gasser O, Schifferli JA. Microparticles released by human neutrophils adhere to erythrocytes in the presence of complement. Exper Cell Res. 2005; 307:381–387. [PubMed: 15950620]
50. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henseon PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature. 2000; 405:85–90. [PubMed: 10811223]
51. Tung JP, Fraser JF, Nataatmadja M, Barnett AG, Colebourne KI, Glenister KM, et al. Dissimilar respiratory and hemodynamic responses in TRALI induced by stored red cells and whole blood platelets [52nd Annual Meeting of the American Society of Hematology]. Blood. 2010; 116(21): 484. Ab#1112.
52. Tung JP, Fung YL, Nataatmadja M, Colebourne KI, Esmaeel HM, Wilson K, et al. A novel in vivo ovine model of transfusion-related acute lung injury (TRALI). Vox Sang. 2011; 100:219–230. [PubMed: 20667072]
53. Bux J, Sachs JJ. The pathogenesis of transfusion-related acute lung injury (TRALI). Br J Haematol. 2007; 136(6):788–799. [PubMed: 17341264]
54. Vandendries ER, Furie BC, Furie B. Role of P-selectin and PSGL-1 in coagulation and thrombosis. Thromb Haemost. 2004; 92(3):459–466. [PubMed: 15351841]
55. Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood. 2006; 108(7):2455–2462. [PubMed: 16772606]
56. Amirkhosravi A, Meyer T, Sackel D, Desai H, Biddinger R, Amaya M, et al. Platelet microparticles upregulate TF and VEGF in endothelial and melanoma cells in a CD40 ligand-dependent manner: Possible role in angiogenesis and metastasis. Blood. 2002; 100(11 Prt II):63b. Ab 3721.
57. Ahn ER, Lander G, Jy W, Bidot C, Jimenez JJ, Horstman LL, et al. Differences of soluble CD40L in sera and plasma: Implications on CD40L assay as a marker of thrombotic risk. Thromb Res. 2004; 114(2):143–148. [PubMed: 15306157]

58. Varo N, Nuzzo R, Natal C, Libbey P, Schonbeck U. Evaluation of soluble CD40L: what works, what does not? [Prepublication manuscript]. Clin Sci. 2006 t.b.a.
59. Jy W, Horstman LL, Wang F, Duncan R, Ahn YS. Platelet factor 3 in plasma fractions: Its relation to microparticle size and thromboses. Thromb Res. 1995; 80(6):471–482. [PubMed: 8610275]
60. Bidot L, Jy W, Bidot C Jr, Fontana V, Horstman LL, Ahn Y-S. Microparticle-mediated thrombin generation assay: enhanced activity in patients with recurrent thrombosis. J Thromb Haemost. 2007; 6(6):913–919. [PubMed: 18363818]
61. Fontana V, Jy W, Dudkiewicz P, Bidot L, Gonzalez M, Yaniz M, et al. Elevated cell-derived microparticles (C-MP) in myeloproliferative disorders (MPD): high red cell microparticles (RMP) as risk factor for thrombosis. Blood. 2006; 108(11):428a. (Ab1483).
62. Zwaal RFA, Comfurius P, Bevers EM. Platelet procoagulant activity and microvesicle formation: Its putative role in hemostasis and thrombosis (Review). Biochim Biophys Acta. 1992; 1180:1–8. [PubMed: 1390938]
63. Shah BS, Beamer N, Coull BM. Enhanced in vivo platelet activation in subtypes of ischemic stroke. Stroke. 1985; 16(4):643–647. [PubMed: 3161220]
64. McGregor L, Martin J, McGregor JL. Platelet-leukocyte aggregates and derived microparticles in inflammation, vascular remodeling and thrombosis [Review]. Frontiers Biosci. 2006 Jan; 11(1): 830–837.
65. Morel O, Jesel L, Freyssinet JM, Toti F. Elevated levels of procoagulant microparticles in a patient with myocardial infarction, antiphospholipid antibodies and multifocal cardiac thrombosis. Thromb J. 2005; 3(15)
66. Bidot L, Jy W, Bidot C Jr, Jimenez JJ, Fontana V, Horstman LL, et al. Microparticle-mediated thrombin generation assay: increased activity in patients with recurrent thrombosis. J Thromb Haemost. 2007; 6:913–919. [PubMed: 18363818]
67. Jy W, Bidot L, Jimenez JJ, Horstman LL, Bang J, Lin A, et al. Thrombin generation profiles are qualitatively and quantitatively distinct in microparticles derived from red cells (RMP), platelets (PMP), and endothelia (EMP). Blood. 2006; 108(11):499a. (Ab1759).
68. Isobe J, Perkmann T, Breuss JM, Binder CJ, Boulanger CM, Binder BR. Red blood cell derived microparticles are thrombogenic in mouse models of atherosclerosis. Blood. 2007; 110(11): 1060A. Ab#3624.
69. Ahlgren E, Lundqvist A, Nordland A, Aren C, et al. Neurocognitive impairment and driving performance after coronary artery bypass surgery. Eur J Cadiothroac Surg. 2003; 23(3):334–3340.
70. Barber PA, Tippett LJ, Frampton C, Milsom P. Postoperative ischemia and cognitive impairment in cardiac surgery patients. Ann Thorac Surg. 2009; 87:672–673. [PubMed: 19161820]
71. Cook DJ, Huston r, Trenerry MR, et al. Postcardiac surgical cognitive impairment in the aged using diffusion-weighted magnetic resonance imaging. Ann Thorac Surg. 2007; 83(4):1389–1395. [PubMed: 17383345]
72. Horstman LL, Jy W, Ahn YS, Zivadinov R, Maghzi AH, Etemadifar M, et al. Role of platelets in neuroinflammation: A wide-angle perspective. J Neurioinflammation. 2010; 7(1):10.
73. Jy W, Horstman LL, Arce M, Ahn YS. Clinical significance of platelet microparticles in autoimmune thrombocytopenias [with Editorial pg 321]. J Lab Clin Med. 1992; 119:334–345. [PubMed: 1583382]
74. Lee YJ, Horstman LL, Janania J, Reyes Y, Kelley R, Ahn YS. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. Thromb Res. 1993; 72:295–304. [PubMed: 8303669]
75. Ahn YS, Horstman LL, Jy W, Jimenez JJ, Bowen B. Vascular dementia in patients with immune thrombocytopenic purpura (ITP). Thromb Res. 2002; 107:337–344. [PubMed: 12565721]
76. VanDijk D, Jansen EWL, Hijman R, et al. Cognitive outcomes after off-pump and on-pump coronary artery bypass graft surgery. JAMA. 2002; 287(11):1405–1412. [PubMed: 11903027]
77. Dewanjee MK, Kapadvanjwala M, Mao WW, Jy W, Ahn YS, Ruzius K, et al. A higher blood flow window of reduced thrombogenicity and acceptable fragmentation in a hollow fiber hemodialyzer. ASAIO J. 1993; 39(3):M363–M367. [PubMed: 8268560]