

Platelet-, leucocyte- and red cell-derived microparticles in stored whole blood, with and without leucofiltration, with and without ionising radiation

Shunnichi Saito¹, Kenneth E. Nollet^{1,2}, Alain M. Ngoma³, Takako Ono¹, Hitoshi Ohto^{1,2}

¹Department of Blood Transfusion and Transplantation Immunology, ²Radiation Medical Science Center, Fukushima Medical University, Fukushima, Japan; ³Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada

Background. Storage lesion, including microparticle formation, has been partially characterised in whole blood, but not in all combinations of pre-storage leucofiltration and/or irradiation.

Materials and methods. Single-donor whole blood products were processed into four subunits: with and without leucofiltration, with and without X-irradiation (25 Gy). Platelet-, leucocyte-, and erythrocyte-derived microparticles and free haemoglobin were measured periodically throughout 42 days of storage.

Results. Pre-storage leucofiltration substantially reduced platelet- and leucocyte-derived microparticle counts throughout storage. Irradiation, in contrast, had no significant effect on microparticle counts. A gate for all microparticles showed a substantial time-dependent increase in unfiltered whole blood. A time-dependent increase in free haemoglobin was greatest in unfiltered, irradiated whole blood.

Discussion. This study indicates that leucofiltration can prevent the formation of leucocyte- and platelet-derived microparticles, and might reduce haemolysis in irradiated whole blood, either by removing factors that provoke haemolysis, or by selective retention of senescent or effete red cells most prone to haemolysis.

Keywords: cell-derived microparticles, leucocyte filtration, irradiation, blood storage, whole blood.

Introduction

Ex vivo changes in labile blood components are collectively referred to as storage lesion, a designation that implies potential harm to transfusion recipients¹⁻³. In contrast, some processing steps, such as leucocyte filtration and irradiation, are intended to benefit transfusion recipients³⁻⁵. In Japanese practice, allogeneic blood components are routinely leucodepleted and irradiated prior to storage. Some early leucocyte filters were associated with specific adverse reactions⁶⁻⁹, but the technology has evolved and pre-storage leucofiltration has emerged as a standard of care in many developed countries. Recent filters can also remove prions^{10,11}. X- or gamma-irradiation of cellular allogeneic blood components, to prevent transfusion-associated graft-versus-host disease, is unequivocally the standard of care in Japan, a country in which the likelihood of a one-way HLA match is exceptionally high¹².

Storage lesion includes cellular changes that result in the formation of extracellular vesicles, including microparticles (MP). MPs also arise naturally in states of health and disease, from endothelial cells,

erythrocytes, leucocytes, megakaryocytes and platelets. In common, MPs are defined as vesicles enclosed by a phospholipid bilayer, with a diameter less than 1,000 nm (1 µm) and more than 50-100 nm. The cell of origin of an MP may be inferred from its surface composition and internal contents, but not to the extent of being a cell's replica-in-miniature. Differences include a loss of membrane asymmetry, with MPs expressing anionic phospholipids normally kept on the inner membrane layer by flippase enzymes. Found in all body fluids, MPs have intercellular signalling and molecular transport functions¹³⁻²⁶.

Platelet-derived microparticles (PDMP) are the most abundant kind of MP naturally present in the circulation, and are thought to have more procoagulant activity than platelets, by one to two orders of magnitude^{24,27}. The relatively fewer red cell-derived microparticles (RDMP) in apheresis platelet components may provoke Rh alloimmunisation²⁸. Other potentially immunogenic red blood cell antigens, such as those of the KEL, JK, FY, MNS, LE and LU systems, have also been detected on microparticles²⁹.

We have previously shown that leucocyte filtration of whole blood reduces platelet concentration and PDMP formation during storage³⁰. Building on previous work, we measured PDMP, RDMP, and leucocyte-derived microparticles (LDMP) in stored whole blood that had or had not been filtered and had or had not been exposed to ionising radiation. Because X and gamma irradiation can provoke the release of haemoglobin (Hb) from red cells³¹, this was also measured during storage.

Materials and methods

Donor selection

Our blood donors were nine healthy males who met the criteria for allogeneic blood collection in Japan. The study protocol was approved by Fukushima Medical University's institutional review board, which is guided by local policy, national law, and the World Medical Association Declaration of Helsinki.

Product collection, processing and storage

Whole blood (400 mL) was collected in Sepacell Integra CA bag sets (ABD-400CA8FL, Asahi Kasei Medical, Tokyo, Japan), each with 56 mL CPDA and an integrated RZ-2000 leucocyte reduction filter. The product

was stored at 2-6 °C for 2 hours, after which half of the total volume was passed through the integrated filter.

As shown in Figure 1, filtered and unfiltered halves were further divided into 100 mL volumes. The resulting four bags were distinguished from each other with a code to indicate filtration and irradiation status (i.e., N00, N25, F00, F25; N=not filtered, F=filtered; 00=0 Gy, 25=25 Gy). Rad-Sure XR 25 Gy irradiation indicators (Ashland, Inc., Covington, KY, USA) were affixed to each bag for subsequent confirmation of the bags having been irradiated or not. Sampling adapters (TC-MP, Terumo BCT, Tokyo, Japan) were attached to each of the four bags, after which day 0 (collection day) aliquots were promptly withdrawn and processed prior to any irradiation, but otherwise as described below, to assess any differences that might have arisen as a result of handling.

After aliquots were withdrawn for day 0 measurements, all four bags were carried in an insulated container to an on-campus facility with an X irradiator (MBR-1520R-3, Hitachi Medical Corporation, Tokyo, Japan). According to how the bags were labelled, one filtered product and one unfiltered product were irradiated with 25 Gy.

Bags were stored for 42 days at 2-6 °C in a walk-in refrigerator.

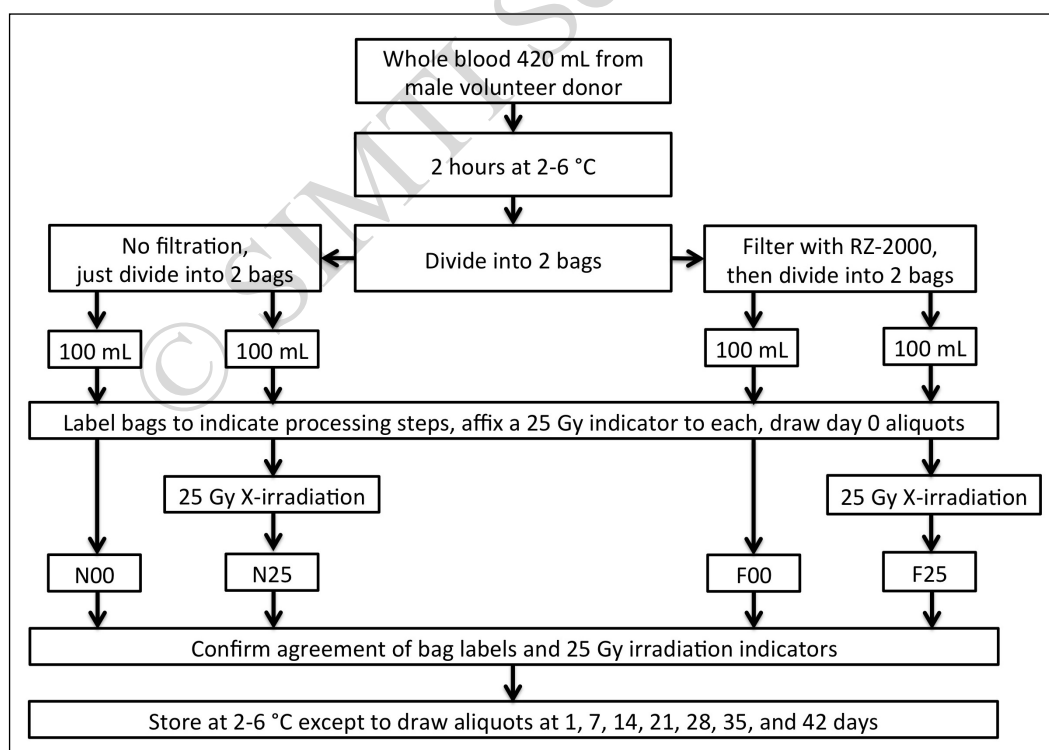


Figure 1 - Study design.

Whole blood units from healthy donors were divided into four bags and processed as shown. Free Hb measurements, not shown on this flowchart, were done in batches on samples taken from frozen storage. N00: not filtered, not irradiated; N25: not filtered, 25 Gy irradiated; F00: filtered, not irradiated; F25: filtered, 25 Gy irradiated.

Periodic withdrawal of test aliquots

Aliquots of 3 mL were drawn on days 0, 1, 7, 14, 21, 28, 35 and 42 of storage. First, bags were removed from refrigeration, gently rocked to mix their contents, and sampling was performed sterily in a positive pressure clean air hood. Aliquots were gently injected into polypropylene test tubes just prior to cell counting on a KX-21 or XP-300 haematology analyser (Sysmex, Kobe, Japan). Promptly thereafter, samples were centrifuged at 2,000 g for 20 minutes. After centrifugation, 250 μ L of each supernatant was transferred to a different tube for same-day flow cytometry; another 500 μ L of each supernatant was transferred to a capped polypropylene storage tube and kept at -80°C for subsequent Hb measurement.

Flow cytometry analysis

Monoclonal antibodies (IOtest, Immunotech, Marseilles, France) were chosen as follows: CD42b-PE (anti-CD42b conjugated to phycoerythrin) to identify PDMP, CD45-PC5 (anti-CD45 conjugated to a tandem dye of phycoerythrin and cyanine 5) to identify LDMP, and CD235a-FITC (anti-CD235a conjugated to fluorescein isothiocyanate) to identify RDMP. Control antibodies were IgG1(mouse)-PE, IgG1(mouse)-PC5, and IgG1(mouse)-FITC.

Simultaneous measurement of PDMP, LDMP, and RDMP proceeded by preparing four positive tubes with 10 μ L each of CD42b-PE, CD45-PC5, and CD235a-FITC; four negative control tubes were prepared with 10 μ L of mouse IgG1-PE, IgG1-PC5, and IgG1-FITC. To each tube so prepared, 50 μ L samples were transferred from corresponding 250 μ L supernatants of not filtered, not irradiated (N00); not filtered, X-irradiated (N25); filtered, not irradiated (F00); and filtered, X-irradiated (F25) products, after which room-temperature incubation proceeded for 20 minutes in darkness. Following incubation, a fixative of 1% paraformaldehyde in phosphate-buffered saline (PBS) was added, after which 4°C fixation proceeded for 30 minutes in darkness. The fixative was prepared fresh on each day with one part filtered 5% paraformaldehyde diluted by four parts reagent-grade PBS. The paraformaldehyde, PBS and freshly prepared fixative were stored at 4°C .

After fixation, 50 μ L of Flow-CountTM Fluorospheres (Beckman Coulter, Fullerton, CA, USA) of known concentration were added to quantify events (per μ L) on a Cytomics FC-500 (Beckman Coulter). Flow cytometry events were classified as MP according to a gating scheme adapted from Nomura and colleagues³², based on fluorescence intensity and the light-scattering properties of size-calibrated polystyrene microspheres (Polysciences, Inc., Warrington, PA, USA).

Statistics

Data were analysed using STATA 12 (StataCorp, College Station, TX, USA). Data were tested for normal distribution by means of the Shapiro-Wilk test. Non-parametric Kruskal-Wallis one-way analysis of variance was used to assess any differences among samples. Statistical significance was set at $p < 0.05$.

Results

Figure 2 shows gating schemes (left, A, C, E) with graphs of PDMP, LDMP, and RDMP as a function of storage time (right, B, D, F) for all four combinations of pre-storage processing.

PDMP counts in non-filtered products increased monotonically by two orders of magnitude through day 35, after which counts remained high, but the monotonic pattern was lost. In contrast to the situation in non-filtered products, PDMP counts in filtered products were lower from the outset, and showed no statistically significant change during 42 days of storage (Figure 2B). This is consistent with the substantial removal of source platelets by leucocyte filters designed for whole blood. At all time-points, PDMP counts among the four differently processed bags were significantly different ($p < 0.05$).

LDMP counts in non-filtered products also increased monotonically throughout 28 days' storage, and remained high thereafter. LDMP in filtered products showed a slight, non-monotonic trend upward with storage, consistent with the fact that most leucocytes were removed by filtration prior to storage, with significant differences of LDMP counts at every time-point from day 7 to day 42 (Figure 2D). Applying the two-sample Wilcoxon rank-sum (Mann-Whitney) test to PDMP and LDMP, significant differences were observed between filtered (F00, F25) and non-filtered (N00, N25) products, but no statistically significant differences emerged between non-irradiated (F00, N00) and irradiated (F25, N25) products at any time-point from day 7 to day 42.

With red cells as the dominant cellular component of whole blood, the baseline number of RDMPs increased substantially during storage, regardless of filtration or irradiation status. After day 21, RDMP counts of non-filtered products did not increase to the extent of that of filtered products, and, in the case of N00, the pattern was not monotonic after day 21 (Figure 2F). No statistically significant differences in RDMP counts could be attributed to either filtration or irradiation at any time-point. Applying the two-sample Wilcoxon rank-sum (Mann-Whitney) test to RDMP, no significant differences were observed between filtered and non-filtered (F25 vs N25 and F00 vs N00), or irradiated and non-irradiated (F00 vs F25 and N00 vs N25) products, at any time-point from day 0 to day 42.

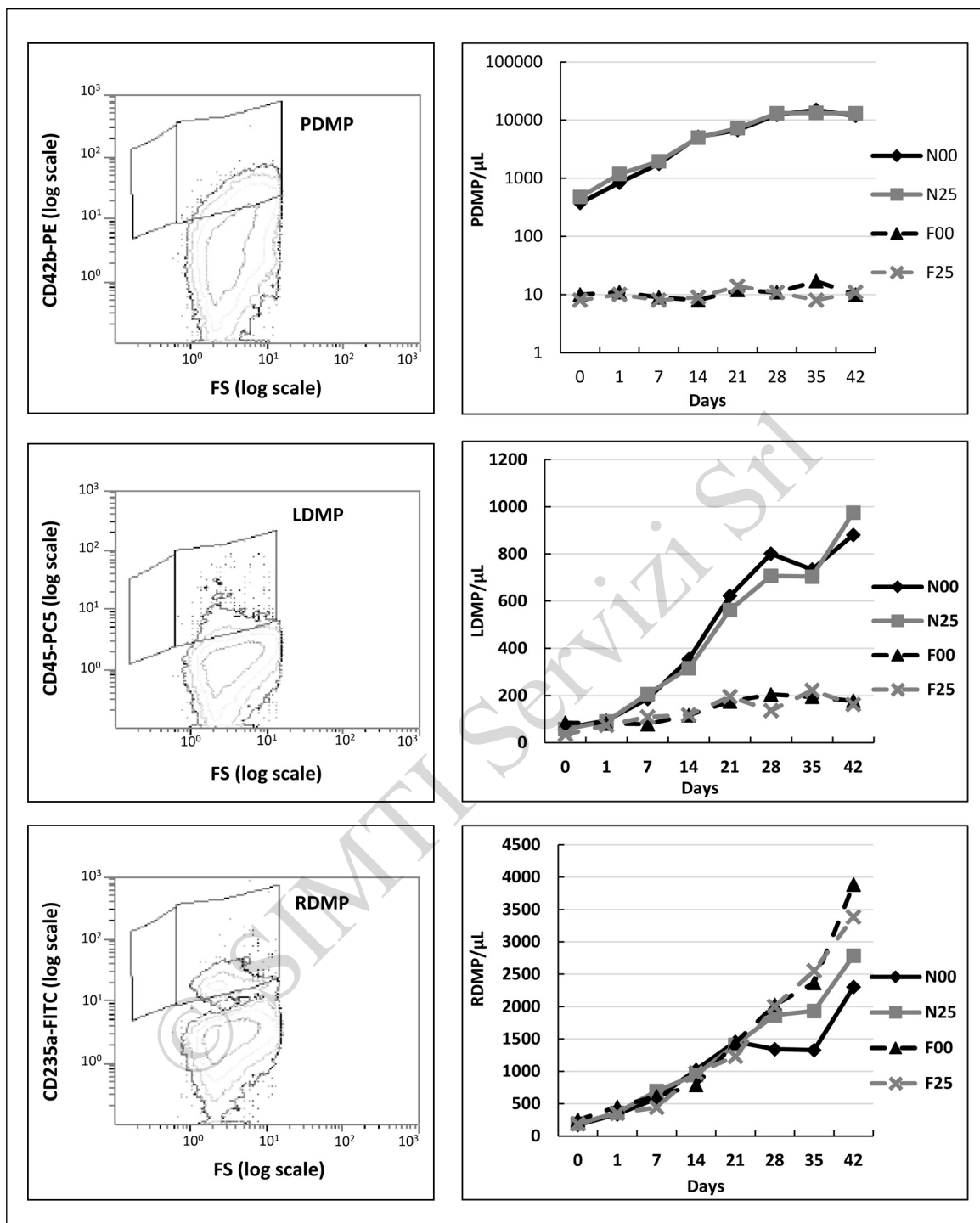


Figure 2 - Gating schemes (left: A, C, E) and microparticle counts (right: B, D, F) at 0, 1, 7, 14, 21, 28, 35 and 42 days. Top (A, B): platelet-derived microparticles (PDMP). Middle (C, D): leucocyte-derived microparticles (LDMP). Bottom (E, F): red cell-derived microparticles (RDMP). Gating schemes for PDMP, LDMP, and RDMP were designed to identify microparticles (MP) from 0.5 to 1.0 μm by calibration with standard 0.5, 1.0, and 2.0 μm diameter polystyrene beads. Adjustments along the vertical axis were to accommodate variations of fluorescence intensity among the reporter antigens. AA, AC and AG gates capture non-specific reactions. Line graphs show the progress of MP formation during storage at 4 °C, as affected by leucoreduction and/or irradiation. N00: not filtered, not irradiated; N25: not filtered, 25 Gy irradiated; F00: filtered, not irradiated; F25: filtered, 25 Gy irradiated.

Figure 3 shows free Hb changes during storage. As expected, statistically significant increases in free Hb occurred with time regardless of pre-storage processing. Although differences among pre-storage processing conditions did not achieve statistical significance, the free Hb trend line of bags not filtered but irradiated (N25) rose distinctly above that of the other three comingled lines after day 14.

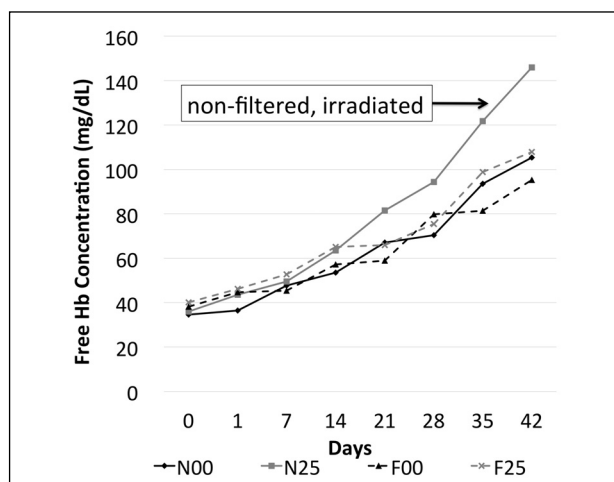


Figure 3 - Free haemoglobin (Hb) concentrations at 0, 1, 7, 14, 21, 28, 35 and 42 days. N00: not filtered, not irradiated; N25: not filtered, 25 Gy irradiated; F00: filtered, not irradiated; F25: filtered, 25 Gy irradiated. Irradiated (F25 and N25) products tended to have higher free Hb than non-irradiated products (F00 and N00), but not to the point of statistical significance.

We also gated for all events below $1.0 \mu\text{m}$, with no lower cut-off and with no regard to fluorescent antigen binding (Figure 4), thus including MPs of any origin. Counts during storage followed the general pattern of the PDMP gate, but were higher by an order of magnitude. From day 21, the visually apparent difference between unfiltered and filtered products achieved statistical significance ($p < 0.05$).

Figure 5 (top: A, B) shows CD235a positivity, characteristic of RDMP, within the PDMP gate. Absolute counts in this combination gate trended upwards during storage in unfiltered products, in contrast to seemingly random fluctuations around the day 0 baseline in filtered products. Even so, these CD235a-positive events accounted for a near-zero percentage of PDMP-gated counts throughout the 42 days of storage in unfiltered products compared to about half of the PDMP-gated counts in filtered products. Although artefacts cannot be dismissed, it is plausible that PDMPs, to the extent that they are available (more in unfiltered products, less in filtered products), are potential targets for antigen transfer from an overwhelming population of

RDMPs. The much smaller number of PDMPs in filtered products would, individually, have more antigen transfer encounters with RDMPs, so that a larger fraction of them would fall into the RDMP fluorescence intensity gate.

Figure 5 (middle: B, C) shows CD42b positivity, characteristic of PDMP, within the LDMP gate. PDMP-positive counts in the LDMP gates of N00 and N25 products increased until at least day 28, with irregular results thereafter. PDMP-positive counts remained relatively flat, below $10/\mu\text{L}$, in the LDMP gates of F00 and F25 products. PDMP positivity as a percentage of the LDMP gates tended downwards during storage in F00 and F25 bags, with random fluctuations over time in the N00 and N25 bags. It stands to reason that filtration, which reduces both platelet and leucocyte counts, would result in low absolute counts of microparticles carrying both platelet and leukocyte antigens. In the absence of filtration, time affords more opportunity for antigen-transfer events, and thus, higher absolute counts in unfiltered products. Seemingly random changes in the percentage of PDMP-positive LDMP, with or without any time-dependent trend, imply variables that are yet to be determined.

RDMP-positive counts in the LDMP gate trended upward to the same degree and in the same range among the N00, N25, F00, and F25 bags (Figure 5E), consistent with the expectation that time affords more opportunity for antigen-transfer events. Differences emerged in the fraction of RDMP positivity (Figure 5F). On day 0, regardless of filtration or irradiation status, 60-80% of LDMP-gated events were also positive for RDMP. In unfiltered products, this percentage fell during storage

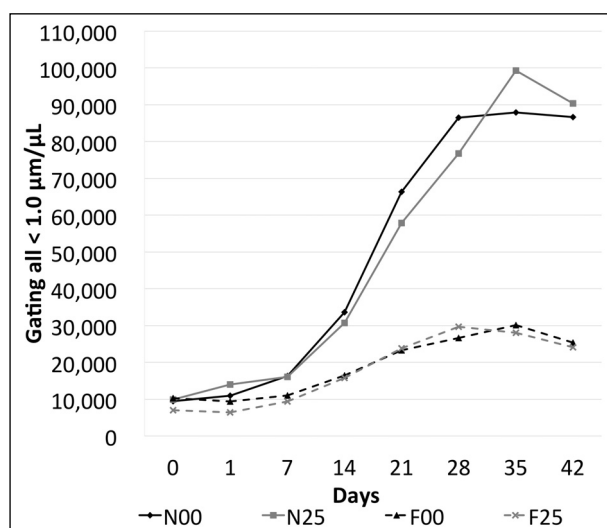


Figure 4 - All MP $< 1.0 \mu\text{m}$, with or without fluorescent antigen binding, at 0, 1, 7, 14, 21, 28, 35 and 42 days. N00: not filtered, not irradiated; N25: not filtered, 25 Gy irradiated; F00: filtered, not irradiated; F25: filtered, 25 Gy irradiated.

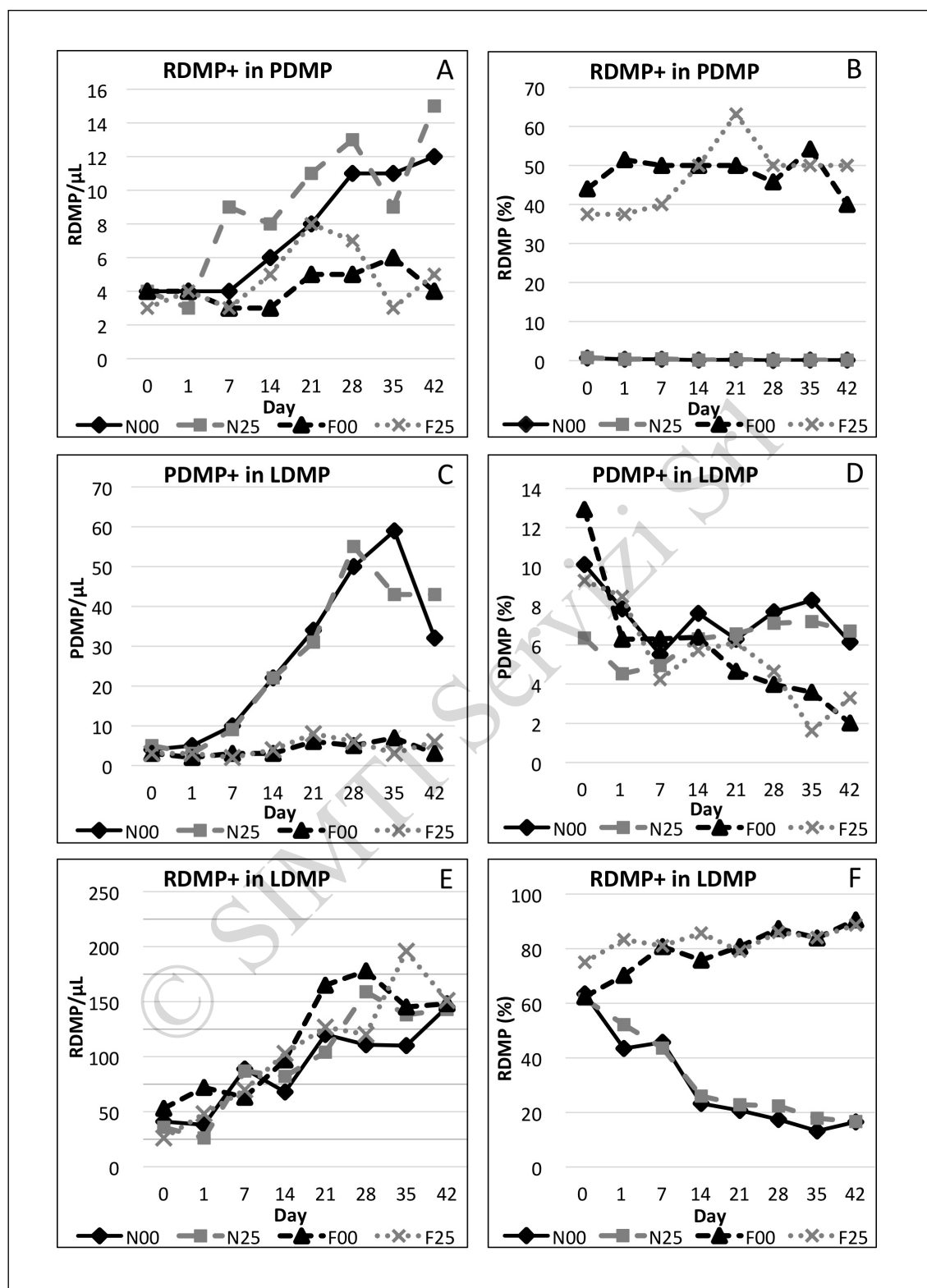


Figure 5 - Concentrations (left: A, C, E) and percentages (right: B, D, F) of shared-antigen MP at 0, 1, 7, 14, 21, 28, 35 and 42 days.

Top (A, B): RDMP-antigen-positive PDMP. Middle (C, D): PDMP-antigen-positive LDMP. Bottom (E, F): RDMP-antigen-positive LDMP. N00: not filtered, not irradiated; N25: not filtered, 25 Gy irradiated; F00: filtered, not irradiated; F25: filtered, 25 Gy irradiated; PDMP: platelet-derived microparticles; LDMP: leucocyte-derived microparticles; RDMP: red cell-derived microparticles.

to less than 20% by days 35 and 42. Filtered products, with a relative paucity of LDMPs, showed an upwards trend, with >80% RDMP-positivity in the LDMP gate by days 28–42. As with PDMPs, the much smaller number of LDMPs in filtered products would, individually, have more antigen transfer encounters with RDMPs, so that a larger fraction of them would fall into the RDMP fluorescence intensity gate.

Discussion

We investigated storage lesion over 42 days in whole blood that had undergone pre-storage leucocyte filtration (F) or not (N), followed by X-irradiation (25 Gy) or not (00 Gy). Periodic measurements included cell counts with a haematology analyser, MP counts determined by flow cytometry, and free Hb concentration assessed by optical density. PDMP and LDMP counts were significantly higher in non-filtered products (N00 and N25) than in filtered (F00 and F25) products, whereas RDMP counts did not seem to be affected by either filtration or irradiation. In fact, irradiation did not appear to affect MP generation at any time during storage.

Free Hb concentration was analysed as another indicator of storage lesion. Irradiated, non-filtered products (N25) tended to release more Hb into solution than other products, but not to a level of statistical significance. Other reports^{33,31} described statistically significant increases in free Hb over time in irradiated vs non-irradiated products. The lack of statistical significance in our study may be related to sample size in this instance, but the data nevertheless suggest that leucofiltration may reduce storage-associated haemolysis, either by removing factors that provoke haemolysis or by selective retaining red cells most prone to haemolysis. Consistent with the possibility of some benevolent synergy between irradiation and filtration, Nagahashi *et al.* reported that combined filtration and irradiation did not affect serum K⁺, pH or ATP in red blood cells, compared with filtration or irradiation alone³⁴. Sawa *et al.* reported that free Hb concentrations after filtration were significant higher than free Hb concentrations without filtration³³. Seemingly at odds with our findings, this invokes the possibility that some filters, rather than reducing subsequent haemolysis, might damage red cells in transit. Various factors may account for such differences, including the type of blood component (e.g., whole blood vs red blood cells), how products are filtered (e.g., time and temperature of filtration, filter surface chemistry and geometry), as well as whether and how products are irradiated. Considering the wide range of values measured within each type of product from our cohort, yet-to-be-defined factors may also contribute.

RDMP increased similarly with or without pre-storage filtration. *In vivo*, RDMPs are reported to increase in response to inflammatory cytokines, post-operative thrombosis, and transfusion-related acute lung injury^{35–37}. Kriebardis *et al.* wrote that RDMPs improve the survival of transfused red blood cells, however they might enhance adverse events related to microcirculatory dysfunction³⁵. In addition, it has been reported that RDMPs are associated with red blood cell senescence and apoptosis-like states²⁹. RDMPs generated during blood component storage can scavenge nitric oxide, thus facilitating side effects related to vasoconstriction³⁸. Since RDMP formation does not appear to be mitigated by filtration, more research is warranted to assess the role of RDMPs in transfusion reactions, especially after long periods of storage.

MP formation accompanies a loss of cellular membrane asymmetry that exposes anionic phospholipids with pro-coagulant effects. PDMPs are especially regarded for their efficacy in the benevolent-to-malevolent spectrum of haemostasis and thrombosis²⁷. *In vivo*, PDMPs and LDMPs contribute to the production of biological response modifiers and may, in turn, be stimulated by such modifiers. This raises the spectre of transfusion-related acute lung injury and other inflammatory events being attributable, at least in part, to the MP content of blood components when transfused³⁸.

LDMPs arise from lymphocytes, monocytes, and granulocytes, and are reported to have characteristics derived from their cell of origin. LDMPs derived from T lymphocytes decrease the availability of nitric oxide and increase the oxidative stress of endothelial cells^{23,39}. Monocyte-derived LDMPs carry tissue factor and activate endothelial cell adhesion molecules^{23,39}. It was also reported that high concentrations of monocyte-derived LDMPs may be related to vascular complications of diabetic patients with nephropathy, similar to PDMPs in type 2 diabetes⁴⁰.

Gating for all events below 1.0 μm gives counts one to two orders of magnitude higher than gates that are narrower and/or antibody-specific. Thus, these events evade classification. In a recent report, the haemostatic potential of fresh-frozen plasma produced from whole blood after leucocyte reduction was said to decline⁴¹. Whole blood that was leucofiltered 24 hours after collection had significantly lower counts of such uncharacterised MPs, whereas this was not the case for whole blood filtered after just 6 hours⁴¹. In our study, the storage period before filtration was 2 hours, but filtration significantly decreased MP smaller than 1.0 μm (Figure 4). Differences might be related to the post-filtration storage time at which sampling and MP measurement were conducted. These varied results cast doubt on definitive statements regarding *in vivo* MP function when blood components are transfused.

In recent years, new technologies for MP measurement have been developed. For example, highly sensitive flow cytometric imaging, e.g. the ImageStreamX (Amnis, Seattle, WA, USA) flow cytometer/microscope can detect 0.2 μm diameter MPs⁴². Other high-end flow cytometers also can detect 0.3 μm MPs through the use of W2 mode, which enhances detection resolution in the sub-micron region. Dynamic light scattering, nanoparticle tracking analysis, and other technical advances are also emerging²⁷. Each has its merits and demerits, perhaps raising hopes for a "gold standard" to emerge, while making that gold standard more elusive. We aspire to embrace these technologies, along with traditional markers of clinical significance, such as phosphatidylserine as an indicator of membrane inversions and apoptosis, tissue factor as an indicator of endothelial injury and inflammation, and L-selectin as an indicator of lymphocyte activation. In addition to MP investigations and conventional biochemical assays, kinetic assays and "-omics" techniques are being applied to questions about storage lesion^{2,3}. The inability to do everything in one laboratory may be perceived either as a detriment, or as an opportunity to collaborate with colleagues around the world. We welcome collaboration.

Conclusions

In summary, our data point to the benefit of pre-storage leucocyte filtration as a step to mitigate PDMP and LDMP formation during whole blood storage, and for its potential to minimise Hb release from red cells in irradiated whole blood.

Funding and resources

Asahi Kasei Medical provided the filters used in this research and grant support for related expenses.

Authorship contributions

KEN, SS, and HO designed the study. SS, KEN, TO, and HO participated in donor care and blood collection. SS, KEN, and TO processed blood products and performed laboratory experiments. SS and AMN performed the statistical analysis. All Authors contributed to the composition of the manuscript and approved the submission.

Disclosure of conflicts of interest

HO received grant support and blood collection sets with leucocyte filters from Asahi Kasei Medical. SS, KEN, AMN, and TO declare no conflicts of interest.

References

- 1) D'Alessandro A, Kriebardis AG, Rinalducci S, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. *Transfusion* 2015; **55**: 205-19.
- 2) Oh JY, Stapley R, Harper V, et al. Predicting storage-dependent damage to red blood cells using nitrite oxidation kinetics, peroxiredoxin-2 oxidation, and hemoglobin and free heme measurements. *Transfusion* 2015; **55**: 2967-78.
- 3) Patel RM, Roback JD, Uppal K, et al. Metabolomics profile comparisons of irradiated and nonirradiated stored donor red blood cells. *Transfusion* 2015; **55**: 544-52.
- 4) Bianchi M, Vaglio S, Pupella S, et al. Leucoreduction of blood components: an effective way to increase blood safety? *Blood Transfus* 2016; **14**: 214-27.
- 5) D'Alessandro A. Leucoreduction of blood components: clinical and molecular evidence. *Blood Transfus* 2016; **14**: 212-3.
- 6) Alonso-Echanove J, Sippy BD, Chin AE, et al. Nationwide outbreak of red eye syndrome associated with transfusion of leukocyte-reduced red blood cell units. *Infect Control Hosp Epidemiol* 2006; **27**: 1146-52.
- 7) Alvarado-Ramy F, Kuehnert MJ, Alonso-Echanove J, et al. A multistate cluster of red blood cell transfusion reactions associated with use of a leucocyte reduction filter. *Transfus Med* 2006; **16**: 41-8.
- 8) Fried MR, Eastlund T, Christie B, et al. Hypotensive reactions to white cell-reduced plasma in a patient undergoing angiotensin-converting enzyme inhibitor therapy. *Transfusion* 1996; **36**: 900-3.
- 9) Hume HA, Popovsky MA, Benson K, et al. Hypotensive reactions: a previously uncharacterized complication of platelet transfusion? *Transfusion* 1996; **36**: 904-9.
- 10) Lacroix C, Bougard D, Litaize C, et al. Impact of leucocyte depletion and prion reduction filters on TSE blood borne transmission. *PloS ONE* 2012; **7**: e42019.
- 11) Yokomizo T, Kai T, Miura M, Ohto H. Development of a bifunctional filter for prion protein and leukoreduction of red blood cell components. *Transfusion* 2015; **55**: 330-6.
- 12) Wagner FF, Flegel WA. Transfusion-associated graft-versus-host disease: risk due to homozygous HLA haplotypes. *Transfusion* 1995; **35**: 284-91.
- 13) Tan KT, Tayebjee MH, Lim HS, Lip GY. Clinically apparent atherosclerotic disease in diabetes is associated with an increase in platelet microparticle levels. *Diabet Med* 2005; **22**: 1657-62.
- 14) Nomura S. Function and clinical significance of platelet-derived microparticles. *Int J Hematol* 2001; **74**: 397-404.
- 15) Morel O, Toti F, Bakouboula B, et al. Procoagulant microparticles: "criminal partners" in atherothrombosis and deleterious cellular exchanges. *Pathophysiol Haemost Thromb* 2006; **35**: 15-22.
- 16) Morel O, Toti F, Hugel B, et al. Procoagulant microparticles: disrupting the vascular homeostasis equation? *Arterioscler Thromb Vasc Biol* 2006; **26**: 2594-604.
- 17) Lynch SF, Ludlam CA. Plasma microparticles and vascular disorders. *Br J Haematol* 2007; **137**: 36-48.
- 18) Simak J, Gelderman MP, Yu H, et al. Circulating endothelial microparticles in acute ischemic stroke: a link to severity, lesion volume and outcome. *J Thromb Haemost* 2006; **4**: 1296-302.
- 19) Simak J, Gelderman MP. Cell membrane microparticles in blood and blood products: potentially pathogenic agents and diagnostic markers. *Transfus Med Rev* 2006; **20**: 1-26.
- 20) Magdeleyns EJ, Keuren JF, Curvers J. Factor Va, bound to microparticles released during platelet storage, is resistant to inactivation by activated protein C. *Transfusion* 2007; **47**: 1880-8.
- 21) Keuren JF, Magdeleyns EJ, Bennaghmouch A, et al. Microparticles adhere to collagen type I, fibrinogen, von Willebrand factor and surface immobilised platelets at physiological shear rates. *Br J Haematol* 2007; **138**: 527-33.
- 22) Keuren JF, Magdeleyns EJ, Govers-Riemsag JW, et al. Effects of storage-induced platelet microparticles on the initiation

- and propagation phase of blood coagulation. *Br J Haematol* 2006; **134**: 307-13.
- 23) Lovren F, Verma S. Evolving role of microparticles in the pathophysiology of endothelial dysfunction. *Clin Chem* 2013; **59**: 1166-74.
 - 24) Burnouf T, Chou ML, Goubran H, et al. An overview of the role of microparticles/microvesicles in blood components: Are they clinically beneficial or harmful? *Transfus Apher Sci* 2015; **53**: 137-45.
 - 25) Goubran H, Sabry W, Kotb R, et al. Platelet microparticles and cancer: an intimate cross-talk. *Transfus Apher* 2015; **53**: 168-72.
 - 26) O'Driscoll L. Expanding on exosomes and ectosomes in cancer. *N Engl J Med* 2015; **372**: 2359-62.
 - 27) Johnson L, Raynel S, Seghatchian J, Marks DC. Platelet microparticles in cryopreserved platelets: Potential mediators of haemostasis. *Transfus Apher Sci* 2015; **53**: 146-52.
 - 28) Kitazawa J, Nollet K, Morioka H, et al. Non-D Rh antibodies appearing after apheresis platelet transfusion: stimulation by red cells or microparticles? *Vox Sang* 2011; **100**: 395-400.
 - 29) Canellini G, Rubin O, Delobel J, et al. Red blood cell microparticles and blood group antigens: an analysis by flow cytometry. *Blood Transfus* 2012; **10** (Suppl 2): s39-45.
 - 30) Sugawara A, Nollet KE, Yajima K, et al. Preventing platelet-derived microparticle formation - and possible side effects - with prestorage leukofiltration of whole blood. *Arch Pathol Lab Med* 2010; **134**: 771-5.
 - 31) Janatpour K, Denning L, Nelson K, et al. Comparison of X-ray vs. gamma irradiation of CPDA-1 red cells. *Vox Sang* 2005; **89**: 215-9.
 - 32) Nomura S, Inami N, Fujita S, et al. Proposal of standardization for micro-particle determination by flow cytometer. *Cytometry Res* 2011; **20**: 71-84.
 - 33) Sawa F, Iwasita Y, Motegi S, et al. [Effect of irradiation and leukocyte filtration on red cell transfusion for premature infants in an incubator]. *Jpn J Transfus Med* 1999; **45**: 373-8. [In Japanese, with English abstract.]
 - 34) Nagahashi H, Moriyama S, Tsubokura M, et al. [Effect of a leukocyte removal filter on irradiated RC-MAP]. *Jpn J Transfus Med* 1998; **44**: 322-7. [In Japanese, with English abstract.]
 - 35) Kriebardis A, Antonelou M, Stamoulis K, Papassideri I. Cell-derived microparticles in stored blood products: innocent-bystanders or effective mediators of post-transfusion reactions? *Blood Transfus* 2012; **10** (Suppl 2): s25-38.
 - 36) Jy W, Ricci M, Shariatmadar S, et al. Microparticles in stored red blood cells as potential mediators of transfusion complications. *Transfusion* 2011; **51**: 886-93.
 - 37) Rubin O, Canellini G, Delobel J, et al. Red blood cell microparticles: clinical relevance. *Transfus Med Hemother* 2012; **39**: 342-7.
 - 38) Cognasse F, Hamzeh-Cognasse H, Laradi S, et al. The role of microparticles in inflammation and transfusion: a concise review. *Transfus Apher Sci* 2015; **53**: 159-67.
 - 39) Angelillo-Scherrer A. Leukocyte-derived microparticles in vascular homeostasis. *Circ Res* 2012; **110**: 356-69.
 - 40) Omoto S, Nomura S, Shouzu A, et al. Detection of monocyte-derived microparticles in patients with type II diabetes mellitus. *Diabetologia* 2002; **45**: 550-5.
 - 41) Chan KS, Sparrow RL. Microparticle profile and procoagulant activity of fresh-frozen plasma is affected by whole blood leukoreduction rather than 24-hour room temperature hold. *Transfusion* 2014; **54**: 1935-44.
 - 42) Erdbrugger U, Rudy CK, Etter ME, et al. Imaging flow cytometry elucidates limitations of microparticle analysis by conventional flow cytometry. *Cytometry A* 2014; **85**: 756-70.

Arrived: 22 April 2016 - Revision accepted: 18 July 2016

Correspondence: Kenneth E. Nollet
Department of Blood Transfusion and Transplantation Immunology
Fukushima Medical University
Hikarigaoka 1, Fukushima City
Fukushima 960-1295, Japan
e-mail: nollet@fmu.ac.jp