ABO-immune complex formation and impact on platelet function, red cell structural integrity and haemostasis: an in vitro model of ABO non-identical transfusion


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Background Transfusion of ABO non-identical platelets has been associated with fatal haemolytic reactions, increased red cell transfusion needs and other adverse effects, but the practice of ABO matching in platelet transfusion is controversial. Immune complexes can be formed from the anti-A and/or anti-B antibodies and ABO soluble antigen(s) present in donor and recipient plasma after ABO non-identical transfusions. We hypothesized that these immune complexes affect recipient red cell structural integrity, platelet function and haemostasis.

Study Design and Methods Haemolysis, platelet function and haemostatic function were assessed before and after incubation of recipient red cells, platelets and whole blood with normal saline controls, ABO-identical plasma controls or in vitro-generated ABO-immune complexes.

Results ABO-immune complexes caused significantly increased haemolysis (P < 0.001), inhibition of platelet function (P = 0.001) and disruption of clot formation kinetics (P < 0.005) in both group A and O recipient samples.

Conclusions Substantial changes in platelet function, red cell integrity and haemostasis occur after in vitro exposure to immune complexes. These in vitro findings may explain, in part, previously observed associations of ABO non-identical platelet transfusions with adverse effects including increased red cell transfusion needs, organ failure and mortality.

Key words: haemolysis, immune complex, platelet aggregation, platelet transfusion, thromboelastography, transfusion complications.

Introduction

The traditional concept of blood donor compatibility is based on clinical outcomes of red blood cell (RBC) transfusions, and the term ‘ABO compatible’ is employed to encompass both ‘ABO-identical’ and ‘ABO-compatible (non-identical/minor mismatched)’ RBC transfusions. This has been clinically useful in managing RBC transfusions that are accompanied by minimal amounts of donor plasma.

For example, an AB group recipient would rarely experience adverse effects following transfusion of red cells from ABO non-identical donors. However, except for platelet transfusions, infusion of larger amounts of ABO-incompatible plasma is avoided because of the isoagglutinin content of group O, A and B donor blood [1, 2]. The potential issues with this approach have come with the recognition that transfusing soluble antigen to recipients with the cognate antibody may lead to reduced platelet survival and platelet transfusion refractoriness after repeated ABO non-identical transfusions [3]. It has been proposed that ‘ABO compatible’ be subdivided into ‘identical’ and ‘non-identical’, but this concept has not been widely adopted [2, 4].
RBCs are transfused as a concentrate suspended in small amounts of donor plasma. Both apheresis and whole blood-derived pooled platelets, on the other hand, include an average of about 200–300 millilitres of donor plasma that may contain anti-A and anti-B immunoglobulins as well as A and/or B soluble antigens [5]. A potentially dangerous, but rare side-effect of transfusing ABO non-identical platelets can occur when an A, B or AB group patient receives a non-identical platelet transfusion and exposed to a large amount of potent ABO antibody. It also has been recently proposed that soluble A and B antigens in donor plasma may lead to adverse effects, including pulmonary injury and mortality [1, 6]. Whether ABO antibodies cause injury to recipient endothelial cells, white blood cells, etc. or cause damage through formation of immune complexes with recipient soluble ABO antigen is uncertain. It is established that repeated ABO non-identical platelet transfusions can lead to the accumulation of harmful quantities of circulating ABO-immune complexes [4, 7], presumably due to recipient/donor soluble ABO antigens binding to donor/recipient anti-A and anti-B antibodies.

Observational cohort studies and small, randomized trials have reported the results of ABO-identical and non-identical platelet transfusions. ABO-identical platelet transfusions are associated with better platelet count increments and a decrease in platelet transfusion refractoriness in repeatedly transfused patients with haematologic malignancies and similar disorders [8, 9]. ABO non-identical platelet transfusions are associated with adverse effects in the recipient that cumulatively worsen with increasing number of transfusions [1, 4]. Inaba et al. reported that complication rates, including pulmonary failure and sepsis, were higher after ABO non-identical transfusions given to trauma patients compared to ABO-identical products. Acute respiratory distress syndrome and sepsis were significantly different with higher rates in recipients of ABO ‘compatible’ compared with identical plasma ($P = 0.001$ and $0.02$, respectively). There were non-significant trends towards longer ICU and hospital stays in the recipients of ABO non-identical plasma [1]. Shanwell and colleagues reported that group O recipients of multiple units of ‘universal donor’ AB plasma experienced a significant increase in mortality [2]. Cardiac surgery patients receiving ABO-identical platelet transfusions had shorter lengths of stay and showed a significantly decreased need for red blood cell transfusions [6]. Faster recovery from postoperative fever was also noted in the ABO-identical group [6]. In a small, randomized, controlled trial of 40 acute leukaemia patients, the mean survival for those who reached complete remission was longer for ABO-identical (25 months) than ABO non-identical (13 months) platelet recipients [10]. The potential benefit of transfusing ABO-identical as opposed to ABO non-identical or compatible products has been reported in both medical and surgical settings, but has not been tested in randomized trials except in regard to platelet transfusion refractoriness in patients with haematologic diseases [1, 11]. Recently, an in vitro model of ABO non-identical transfusion demonstrated that when anti-A and anti-B bind to cognate antigens on platelets or in whole blood, functional tests of haemostasis were impaired [12].

The objective of this study is to test the in vitro effects of ABO-immune complexes on platelet function, red blood cell structural integrity and tests of whole blood haemostasis in order to model what might occur in recipients following ABO non-identical transfusions.

Materials and methods

Subjects and blood collection

Venous blood was obtained from healthy volunteers of blood groups A, B, AB and O who were free of any medications known to affect platelet function or haemostasis for a minimum of 1 week. The University of Rochester Research Subjects Review Board granted ethical approval for the protocol, and written consent was provided by all donors. Blood was collected by venipuncture using a 21-gauge needle. In a red top tube, 4 ml of blood was collected and discarded. An additional 24 ml was then collected in 8, 3 ml siliconized Vacutainer tubes containing 3-2% trisodium citrate anticoagulant (Greiner Bio-One, Kremsmünster, Austria) in a 9:1 ratio. Tubes were immediately mixed by gentle inversion.

Blood sample processing

Whole blood processing commenced within 10 min of collection to minimize in vitro platelet activation and modification of complement components and other blood proteins. Two tubes were retained as whole blood for thromboelastography (TEG) analysis. Two tubes were centrifuged at 2500 g for 15 min on an IEC Centra CL2 centrifuge (Thermo Scientific, Waltham, MA, USA). These are similar but not identical to the g forces employed to generate platelet poor plasma for transfusion. Platelet poor plasma (PPP) was collected for concentration and immune complex creation, or to be used as control plasma. The remaining four tubes were centrifuged at 100 g for 12 min to prepare platelet-rich plasma (PRP) for light transmission platelet aggregometry. These are similar but not identical g forces to those used to create platelet-rich plasma for transfusion after further concentration. After PRP collection, the buffy coat was discarded and the red blood cells were diluted in autologous plasma (200 µl) and pooled for haemolysis detection.
Assessment of anti-A and anti-B antibody titres

Anti-A and anti-B isohaemagglutinin titres of all donors (group A, B and O) were determined at baseline, after plasma concentration and again after immune complex formation using tube technique [13, 14]. Briefly, serial twofold dilutions of plasma were prepared from undiluted to 1:2048 using 0.9% saline. Diluted plasma samples were incubated for 15 min at 25°C with one drop of commercially prepared 3% red blood cells possessing the corresponding antigen (Biotestcell A1 & B red blood cells, Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany). The samples were centrifuged for 30 s at 1000 RCF using a Hettich EBA21 centrifuge (Helmer, Noblesville, IN, USA) and evaluated for agglutination. The end-point titre is the highest dilution with + agglutination. All titres were performed by a single individual to reduce variability in titre end-point determinations.

Plasma concentration

Plasma samples of all ABO groups were concentrated under sterile conditions using a Minicon CS15 Protein Concentrator (EMD Millipore, Darmstadt, Germany). In parallel, stocks of group A and O plasma were retained under identical sterile conditions, without concentration, to control for the effects of ageing during storage on plasma proteins. Following storage and prior to use, antibody titres were reassessed for both concentrated and non-concentrated plasmas.

Immune complex formation

Two varieties of immune complexes were formed by mixing equal volumes of concentrated group A and group B (A-B/immune complexes) or group O and group AB (AB-O/immune complexes) plasmas, followed by incubation at 37°C for 30 min with gentle mixing. Complex formation was confirmed by reassessing anti-ABO immunoglobulin titres after incubation (Table 1). Care was taken so that anti-A and anti-B titres after immune complex formation were at or below the proposed ‘critical’ level of 1:64 employed by some laboratories to control for the direct effect of residual, uncomplexed, ABO antibody on cell surface antigens [15].

Free haemoglobin assay

RBC samples (n = 8) from all blood groups (A, B, O and AB; 2 each) were combined at a ratio of 4:1 with normal saline, stored ‘aged’ ABO-identical plasma or the preformed AB-O/immune complexes and incubated at 37°C for 30 min with gentle mixing. Samples were centrifuged at 2500 g for 15 min, and the supernatants were assayed for free haemoglobin concentration using the Quantichrom Hemoglobin Assay Kit (BioAssay Systems, Hayward, CA, USA).

Platelet aggregation assay

Fresh, group A, B, AB and O PRP were prepared and mixed at a ratio of 4:1 with autologous plasma, stored ABO-identical plasma, A-B/immune complex containing plasma or AB-O/immune complex containing plasma and incubated at 37°C for 30 min with gentle mixing. PRP light transmission aggregometry was performed on a Chrono-Log aggregometer (Model 490-20; Chrono-Log Corporation, Havertown, PA, USA). Briefly, 450 µl of PRP samples was distributed into prewarmed glass cuvettes containing stir bars and incubated at 37°C. Stir speed and gain were set at 1200 and 20/5, respectively. Aggregation was induced with 10 µM ADP (Chrono-Lume, Havertown, PA, USA), and data were collected over 20 min. The amplitude was recorded and compared for each sample using Aggrolink software version 5.1.9.

Thromboelastography

Fresh, whole blood samples were combined at a ratio of 4:1 with autologous plasma, stored ABO-identical plasma or AB-O/immune complex containing plasma and incubated at 37°C for 30 min with gentle mixing. Clot kinetics were then assessed on a Thromboelastograph Hemostasis System 5000 Series analyzer (Haemoscope, Niles, IL, USA). Briefly, 1 ml of sample was pipetted into a prewarmed vial of kaolin (Haemoscope) and mixed by gentle inversion. Subsequently, 360 µl of this mixture was added to a prewarmed TEG analyser cup containing 20 µl of 0.2 m calcium chloride (Haemoscope) and analysed for clot kinetics.

Results

Assessment of anti-A and anti-B titres of the concentrated plasmas

Anti-A titres of both group B and O were concentrated to least 1:256. Anti-B titres of both group A and O remained

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at 1:64. Following in vitro immune complex generation, titres of anti-A and anti-B decreased to 1:64 and 1:4, respectively (Table 1).

ABO-immune complexes cause increased red blood cell haemolysis

Free haemoglobin assays were performed to test the in vitro effects of immune complexes on RBCs. An almost 10-fold increase in free haemoglobin levels was detected after the exposure of RBC samples of all ABO groups to AB-O/immune complexes relative to the stored ABO-identical plasma (P < 0.001) (Table 2). Significant haemolysis was also detected in group O RBCs, indicating an immune complex direct lysis effect rather than an antigen-dependent mechanism.

ABO-immune complexes significantly inhibit platelet function

In our experiment, immune complexes formed from a 1:1 mixture of concentrated group A and B plasmas inhibit ADP-induced platelet aggregation independent of blood group (n = 4; one experiment with each blood group) by an average of more than 35% relative to samples treated with autologous plasma (P = 0.001) (Table 3). As our method of concentrating plasma required long exposures of plasma to room temperature, a separate experiment was performed in which the effects of immune complexes on A and O platelet function were compared to those of both autologous and stored, identical ABO group plasma. As seen in Table 4, no changes in ADP-induced aggregation were detected when mixed with ABO-identical plasma that had been stored at ambient conditions. However, ADP platelet aggregation of group A (n = 4) and group O (n = 4) were significantly inhibited (by an average of 70%) when mixed at a ratio of 4:1 with AB-O/immune complex containing plasma in comparison with similarly stored, ABO-identical plasma (P < 0.001 and <0.006, respectively). Controlling for blood group, a more pronounced effect was witnessed in group O platelets than in group A (78% and 63%, respectively) (Table 4).

**In vitro exposure to immune complexes markedly alter clotting kinetics**

Utilizing thromboelastography, exposure of group A (n = 4) and group O (n = 5) whole blood to AB-O/immune complex containing plasma at a ratio of 4:1 led to significant increases (P ≤ 0.005) in fibrin clot initiation time (R time) and time to achieve clot firmness (K time), as well as decreases in the rate of clot formation (ζ angle), absolute clot strength/platelet function measured as maximum amplitude (MA) and overall coagulation index (CI) relative to the control stored, ABO-identical plasma (Table 5). All alterations in clot kinetics suggest an immune complex-driven hypocoguable state as indicated by a mean decrease in the overall CI by almost fourfold (Table 5 and Fig. 1). Controlling for blood group, group O blood whole blood displayed a relatively greater resistance to immune complex-induced reductions in

**Table 2** Free haemoglobin concentration (mg/dl) of all ABO blood groups (2 each, n = 8) after mixing RBC samples with 0.9% NS, stored ABO-identical plasma or in vitro-formed ABO-immune complexes at a ratio of 4:1

<table>
<thead>
<tr>
<th>RBC samples (n = 8)</th>
<th>Mean ± SD (mg/dl)</th>
<th>Range (mg/dl)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC + NS (0.9%)</td>
<td>20 ± 9</td>
<td>10–30</td>
<td>–</td>
</tr>
<tr>
<td>RBC + stored</td>
<td>10 ± 8</td>
<td>0–22</td>
<td>0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ABO-identical plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC + in vitro-formed immune complexes</td>
<td>97 ± 16</td>
<td>77–131</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>In comparison with 0.9% normal saline.
<sup>b</sup>In comparison with the stored ABO-identical plasma (Student’s t-test).

**Table 3** ADP-induced platelet aggregation (%) of ABO groups (1 each; n = 4) mixed at a ratio of 4:1 with autologous plasma or A-B/immune complexes (generated in vitro from A and B plasmas)

<table>
<thead>
<tr>
<th>ADP PRP Aggregation</th>
<th>Mean ± SD (%)</th>
<th>Range (%)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline PRP aggregation</td>
<td>84 ± 8.0</td>
<td>79–96</td>
<td>–</td>
</tr>
<tr>
<td>PRP + autologous plasma</td>
<td>67 ± 6.4</td>
<td>63–77</td>
<td>–</td>
</tr>
<tr>
<td>PRP + in vitro-formed A-B/immune complexes</td>
<td>44 ± 4.2</td>
<td>38–48</td>
<td>0.001</td>
</tr>
</tbody>
</table>

PRP: platelet-rich plasma.

<sup>a</sup>P value is compared with autologous plasma (Student’s t-test).

<table>
<thead>
<tr>
<th>ADP PRP Aggregation</th>
<th>Group A (n = 4) Mean ± SD (%)</th>
<th>Group O (n = 4) Mean ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline PRP aggregation</td>
<td>91 ± 4</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>PRP + autologous plasma</td>
<td>84 ± 14</td>
<td>85 ± 15</td>
</tr>
<tr>
<td>PRP + stored ABO-identical plasma</td>
<td>83 ± 13</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>PRP + in vitro-formed A-B/O/immune complexes</td>
<td>31 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19 ± 21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

P value<sup>a</sup> <0.001, <sup>b</sup>0.006 in comparison with the stored ABO-identical plasma (Student’s t-test).
Table 5 Alteration of TEG clot kinetic parameters of whole blood samples of group A (n = 4) and group O (n = 5) (group A only, Table 5B or group O only, Table 5C) normal donors after mixing at a ratio of (4:1) with autologous plasma, stored ABO-identical plasma or in vitro-formed AB-O/immune complexes

<table>
<thead>
<tr>
<th>Tested samples (n = 9)</th>
<th>TEG parameters (mean ± SD)</th>
<th>R time</th>
<th>K time</th>
<th>Angle</th>
<th>MA</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> Baseline WB TEG</td>
<td>6.0 ± 0.8</td>
<td>3.4 ± 1.1</td>
<td>50 ± 9</td>
<td>55 ± 5</td>
<td>−2.4 ± 2</td>
<td></td>
</tr>
<tr>
<td>WB + autologous plasma</td>
<td>6.9 ± 1.7</td>
<td>3.2 ± 1.2</td>
<td>53 ± 10</td>
<td>55 ± 4</td>
<td>−2.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>WB + stored ABO-identical plasma</td>
<td>6.3 ± 0.9</td>
<td>2.6 ± 0.7</td>
<td>59 ± 7</td>
<td>57 ± 3</td>
<td>−1.6 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>WB + in vitro-formed AB-O/immune complexes</td>
<td>8.6 ± 1.6a</td>
<td>4.7 ± 1.6b</td>
<td>42 ± 8c</td>
<td>50 ± 5b</td>
<td>−6.5 ± 3.1b</td>
<td></td>
</tr>
<tr>
<td><strong>B: Group A (n = 4)</strong> Baseline WB TEG</td>
<td>5.4 ± 0.8</td>
<td>3.1 ± 1</td>
<td>53 ± 8</td>
<td>55 ± 5</td>
<td>−1.7 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>WB + autologous plasma</td>
<td>6.5 ± 2</td>
<td>3.3 ± 1.5</td>
<td>53 ± 13</td>
<td>54 ± 5</td>
<td>−2.6 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>WB + stored ABO-identical plasma</td>
<td>5.9 ± 0.8</td>
<td>2.4 ± 0.6</td>
<td>60 ± 7</td>
<td>57 ± 2</td>
<td>−1.1 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>WB + in vitro-formed AB-O/immune complexes</td>
<td>9.3 ± 2.3a</td>
<td>5.7 ± 1.1b</td>
<td>37 ± 4b</td>
<td>45 ± 3c</td>
<td>−7.6 ± 2b</td>
<td></td>
</tr>
<tr>
<td><strong>C: Group O (n = 5)</strong> Baseline WB TEG</td>
<td>6.4 ± 0.4</td>
<td>3.7 ± 1.3</td>
<td>48 ± 10</td>
<td>55 ± 5</td>
<td>−2.9 ± 2</td>
<td></td>
</tr>
<tr>
<td>WB + autologous plasma</td>
<td>7.3 ± 1.6</td>
<td>3.2 ± 1</td>
<td>52 ± 8</td>
<td>56 ± 4</td>
<td>−2.9 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>WB + stored ABO-identical plasma</td>
<td>6.7 ± 0.9</td>
<td>2.7 ± 0.8</td>
<td>57 ± 7</td>
<td>56 ± 4</td>
<td>−2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>WB + in vitro-formed AB-O/immune complexes</td>
<td>8.0 ± 0.7a</td>
<td>3.9 ± 1.6</td>
<td>45 ± 9b</td>
<td>53 ± 3</td>
<td>−5.6 ± 3.8</td>
<td></td>
</tr>
</tbody>
</table>

WB, whole blood; MA, maximum amplitude; CI, coagulation index.

*p value < 0.05, * *p value < 0.005, * * *p value < 0.001 in comparison with the stored ABO-identical plasma (Student’s t-test).

Fig. 1 A combination of three TEG traces for group A and group O whole blood samples at baseline, after treatment at a ratio of 4:1 with stored ABO-identical plasma or AB-O/immune complexes.
measures of haemostasis than did group A. Individually, group A whole bloods displayed significant clotting deficiencies in every metric, while only R time and angle reached significance in group O samples despite an overall trend towards altered kinetics for all parameters.

Discussion

In these studies, we modelled post-transfusion recipient conditions following ABO non-identical platelet transfusion by mixing of in vitro immune complexes with fresh donor RBCs and plasma. This mixture contained ABO-immune complexes, complement, soluble A and B antigens and ABO antibodies. All samples were treated at a ratio of 4:1 to represent a transfusion of roughly three to four transfusions of platelets to an adult patient, or proportionally equal for paediatric patients. Many patients receive these large doses of platelets over the course of a few days. Our findings show that in vitro exposure to ABO-immune complexes leads to defects in RBC and platelet functions and alterations in overall haemostasis.

Case reports of haemolysis due to platelet transfusions containing ABO-incompatible plasmas have been documented with donor anti-A and anti-B antibody titres ranging from 1:32 to well above 1:1024 [16]. Our titering techniques were similar to those employed in clinical laboratories worldwide and in the literature [13, 14]. European policy for the prevention of haemolytic transfusion reactions after ABO non-identical platelet transfusions has established 1:64 as the ‘critical’ value, or maximal titre that can be safely transfused [17, 18]. This cut-off is not based on strong clinical outcomes evidence but is widely accepted. In this study, anti-A and anti-B titres were on the lower end of this range and close to those considered safe for transfusion. Nonetheless, significant alterations in cell and haemostatic function were detected in our experiments. As clinical experience and some experimental data suggest, higher donor ABO titres are associated with more adverse effects in the recipient, our results might underscore the in vivo effect of ABO non-identical transfusions in some cases [12, 14]. Other important issues, such as antibody affinity and biologic potency at fixing complement, amongst others, may play important roles but are not easily modelled.

The mean free haemoglobin concentration of our moderately concentrated, immune complex-treated RBC samples (97 mg/dl) approximates values associated with haemoglobinuria, abdominal pain, increased blood pressure, jaundice and dysphagia in patients [19]. This finding provides one possible explanation for the observation that trauma, surgical and leukaemia patients transfused with ABO non-identical platelets have an increased need for red blood cell transfusions [6, 11, 12]. Immune complex-driven RBC haemolysis is a known complication in ABO-incompatible transfusions [2, 20]. This is thought to be due to interaction between immune complexes and the complement system [21, 22]. Upon antigen binding, immunoglobulins demonstrate a change in their Fc domain that greatly increases complement C1q receptor affinity [23]. Bound C1q proteolytically activates a cascade of zymogen precursors, resulting in the assembly of a C5b-9 membrane attack complex that integrates into and damages target cell membranes [22, 24]. Platelets are also susceptible to immune destruction and may be particularly prone to ‘bystander destruction’, whereby membrane attack complexes non-specifically injure nearby cells [25, 26]. There is also growing evidence implicating direct platelet interaction with complement proteins as an important regulator of the classical and alternative pathways [27, 28]. Platelet C1q-R and gC1q-R receptors are responsible for 40–50% of the peripheral blood cell-bound C1q after in vitro exposure of anticoagulated blood to monomeric and complexed C1q [29]. Furthermore, platelets express myriad proteins involved in cell adhesion to macrophages and neutrophils that initiate inflammatory responses leading to rapid platelet clearance from the circulation [30, 31]. P-selectin is an example of a secreted membrane receptor involved in intracellular communication that may also activate the alternative complement pathway [5, 23]. Unwanted cell clearance can also result from the platelet-mediated removal of circulating immune complexes. Worth et al. used flow cytometry and electron microscopy to show that IgG complexes are bound and endocytosed by platelets through the FcγRIIA receptor and then cleared by association with professional phagocytes [32]. This mechanism is linked to the premature destruction of platelets in disease states such as idiopathic thrombocytopenic purpura [33, 34]. In addition, it is known that ABO-immune complexes adhere to platelet FcγRIIA with specificity as this is inhibited by anti-FcγRIIA monoclonal antibody [33].

Here, we demonstrated that ABO-immune complexes in the presence of complement proteins have a marked effect on platelets. In a previous study, it was found that group O donor plasma containing moderate to high titres of anti-A and anti-B antibodies inhibits platelet response to ADP in group A PRP [12]. This was likely due to direct immunoglobulin binding to platelet surface A antigen [12]. The present study controlled for direct antigen–antibody surface binding with the inclusion of group O platelets. The significant inhibition of platelet light transmission aggregation witnessed in both group A and group O PRP after treatment support an immune complex-mediated decrease in platelet function.

George et al. reported that inflammation plays a key role in the induction of bleeding in connection with low
platelet counts. In an animal model, immune complexes caused massive petechial bleeding in thrombocytopenic mice but not in the untreated control mice or mice treated with non-immune IgG [35]. We observed significant inhibition of clot formation kinetics measured by TEG in ABO-immune complex-treated whole blood samples relative to stored plasma control. ABO-immune complex effects mirrored those seen in thrombocytopenic patients, R time prolongation and reduced MA [36].

The difference between group 0 and group A TEG analyses suggests roles for both surface ABO antigen–antibody interaction and ABO-immune complex formation as contributors to overall haemostatic effects [12]. This is not surprising considering that cell lysis as a result of direct ABO immunoglobulin-surface antigen binding is well characterized and serves as the basis for the longstanding practice of ABO matching in RBC transfusions. While the unbound ABO antibody in our AB-O/immune complex plasmas was present at a titre commonly perceived as less dangerous (<1:64), the combination of large quantities of immune complexes and residual free antibody may be particularly potent under the conditions we employed. We previously reported haemostatic inhibition of group A whole blood TEG metrics and platelet function after exposure to 0 plasma with a moderate anti-A titre (1:128) and no inhibition after control ABO-identical plasma treatment [12]. It is important to recognize the limitations of this study. We cannot be certain how accurately we modelled typical in vivo conditions by controlling for the effects of immune complex alone on the samples. Transfused products contain many different antibodies, soluble antigens and other proteins. The same is true of our in vitro-generated immune complex plasma mixtures. We hypothesize that some of the effects we observed as immune complex mediated since these effects were not seen after exposure to control plasmas without antibody or immune complexes. However, matrix effects or other effects of mixing plasmas cannot be ruled out completely. A variable that was difficult to model was antibody titre. By concentrating samples to reach critically high values, we may have altered complement and other protein concentrations due to their instability at room temperature. We attempted to correct for this by the inclusion of a stored plasma control and by treating fresh samples that would not be storage modified. It will be useful to more accurately model clinical conditions by using non-concentrated antibody containing plasmas in future experiments.

In conclusion, exposure of red cells, platelets and whole blood to plasma containing ABO-immune complexes formed in vitro significantly affects laboratory measures of haemolysis, platelet and whole blood haemostatic function, as compared with ABO-identical plasma controls. This was demonstrated for all ABO groups, suggesting a direct role of ABO-immune complexes on RBC integrity and platelet function. We hypothesize that immune complexes formed in vivo after ABO non-identical transfusion may lead to clinical outcomes including haemolysis, platelet dysfunction and haemostasis alterations. ABO-identical transfusions might reduce the risk of these proposed complications and improve patient outcomes, but randomized clinical trials will be necessary to assess these hypotheses.

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Conflict of interest

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

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