

# Platelet Transfusion Induces Alloimmunization to D and Non-D Rhesus Antigens

Johanna Reckhaus<sup>a</sup> Markus Jutzi<sup>b</sup> Stefano Fontana<sup>b</sup> Vera Ulrike Bacher<sup>a</sup> Marco Vogt<sup>a</sup>  
Michael Daslakis<sup>a</sup> Behrouz Mansouri Taleghani<sup>a</sup>

<sup>a</sup>University Department of Hematology, Inselspital, University of Bern, Bern, Switzerland;

<sup>b</sup>Interregional Blood Transfusion Swiss Red Cross, Bern, Switzerland

## Keywords

Platelet transfusion · Rhesus alloimmunization · Hemovigilance

## Summary

**Background:** Platelet concentrates (PC) contain residual contaminating red blood cells (RBC), being higher in pooled buffy coat PC (BC-PC) than in apheresis units (AP-PC). Data about PC-induced alloimmunization against non-D Rhesus (Rh) antigens are limited. **Methods:** For all newly detected RhD and non-D alloantibodies between August 2015 and September /2017 we prospectively evaluated if they were triggered through PC by analyzing for incompatible RBC and/or PC transfusions. **Results:** We found 5,799 positive results in 89,190 antibody screening tests. We identified 13 newly detectable Rh antibodies through incompatible PCs in 11 patients: 6× anti-D, 4× anti-E, 2× anti-c, 1× anti-f. They received a total of 156 PC (83 BC-PC; 73 AP-PC): 5 patients received incompatible BC-PC only, 1 patient received incompatible AP-PC only, 5 patients received incompatible BC-PC and AP-PC. Quality control showed a mean (range) of 0.304 (0.152–1.662) and 0.014 (0.003–0.080) × 10<sup>9</sup> RBC/l for BC-PC and AP-PC, respectively. Ten of the 11 patients received RBC transfusions, all of them being antigen-negative for the alloantibodies identified. **Conclusions:** PC transfusions may not only induce RhD alloimmunization, but also immunization against further Rh antigens such as c, E, and f. The risk seems higher for BC-PC than for AP-PC. The results may have impact on future recommendations of PC transfusion with respect to Rh compatibility and upper limits of RBC contamination.

© 2018 S. Karger GmbH, Freiburg

## Introduction

Platelet concentrates (PC) may be either obtained by apheresis procedure from one donor (AP-PC) or may be prepared by pooling the buffy coats (BC-PC) or the platelet-rich plasma of 4–6 whole blood donations [1]. In Switzerland, only AP-PC and BC-PC are available. Our regional blood transfusion services usually have a mix of both types of PC in order to optimize the use of whole blood donations, to mitigate the risk of shortages, and to ensure adequate availability of HLA-typed AP-PC. Since the introduction of mandatory universal pathogen inactivation of all PC in 2011 [2], AP-PC and BC-PC are considered clinically equivalent. However, some differences exist between both types of PC, such as the extent of residual contaminating red blood cells (RBC), which has been shown to be considerably lower for AP-PC (0.00017–0.009 ml) compared to BC-PC (0.036–0.59 ml) [3–8].

In comparison to RBC transfusion, where up to 80% of immunocompetent Rhesus D(RhD)-negative individuals may develop serologically detectable anti-D within 2–5 months after exposition to RhD-positive RBCs [9–13], RhD alloimmunization after PC transfusion is rather uncommon. Earlier studies reported the incidence of D alloimmunization in D-negative recipients of D-positive PC transfusions to be up to 19% [3, 14–18]; more recent studies revealed lower frequencies of 0–7% [19–23].

Anti-D alloantibodies often may cause severe hemolytic disease of D-positive fetuses/newborns. They also preclude for the recipients to receive D-positive RBC in times of D-negative RBC shortage. Based on the reported frequencies of D alloimmunization in some recent large studies, the administration of Rh Immune Globulin (RhIg) is recommended, if D-mismatched PC prepared from whole blood donations are transfused to D-negative recipients, particularly to females of childbearing potential [8].

**Table 1.** Demographic and clinical characteristics of the 11 patients

Patient-#	Age / sex	Cause of hospitalization	Therapy resulting in reduced immune function	ABO group	Rh phenotype	Pregnancy
1	63 / m	angioblastic T-cell lymphoma	CHOEP chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone, etoposide)	A	CCD.ee	–
2	80 / f	biologic aortic valve replacement	–	A	ccddee	not known
3	73 / f	acute myeloid leukemia	cytarabine, daunorubicin	B	CCD.ee	yes (3)
4	53 / m	metastatic rectum carcinoma	FOLFOX chemotherapy (oxaliplatin, folinic acid, 5-fluorouracil)	0	ccddee	–
5	71 / f	aortic dissection and aortic insufficiency	–	A	ccddee	not known
6	78 / f	thrombocytopenia of unknown origin	–	AB	ccddee	not known
7	64 / m	multiple myeloma	high-dose chemotherapy (bendamustine + melphalan), autologous stem cell transplantation	A	CCD.ee	–
8	61 / f	acute promyelocytic leukemia	As-trans retinoic acid and arsenic trioxide	A	ccddee	not known
9	56 / m	carcinoma testis	high-dose chemotherapy with carboplatin and etoposide	A	CcD.ee	–
10	76 / m	AML, upper gastrointestinal bleeding	decitabine	A	ccddee	–
11	65 / m	acute myeloid leukemia	cytarabine, daunorubicin	0	CCD.ee	–

There is some evidence that the minimum quantity of RBCs necessary for primary immunization is as low as 0.03–0.05 ml [8, 24, 25]. Therefore, a presumed advantage of AP-PC is their lower RBC content, which is being considered as insufficient for triggering an alloimmunization. On this basis, some centers do not routinely provide RhIg prophylaxis after administration of RhD-positive AP-PC to RhD-negative patients [8].

On the other hand, Kitazawa et al. [24] hypothesized whether also RBC-derived microparticles could trigger alloimmunizations. They estimated that AP-PC contain 0.0001–0.001 ml of RBC-derived microparticles.

Rh antigens c, C, e and E are less immunogenic than D [13]. So far, there is limited data on PC-induced alloimmunization against other Rh antigens than D [7, 19] and incompatibilities of non-D Rh antigens have generally been disregarded in BC-PC or AP-PC transfusion [8].

In the context of preparing the hemovigilance report to Swissmedic in 2015, the work-up of a newly formed alloantibody revealed an anti-f specificity, which must have been triggered by RBC antigen exposure through an AP-PC transfusion. This prompted us to implement a systematic prospective assessment of every single newly identified Rh alloantibody for the putative triggering blood product transfused. With this hemovigilance-induced quality assurance measure, we aimed to identify possible further cases in order to delineate the frequency, the antibody specificities, and the circumstances that may be associated with this phenomenon. We here describe 13 Rh antibodies detected between August 2015 and September 2017 in 11 patients, where Rh alloimmunization has obviously been provoked by platelet transfusion. Furthermore, we compared the frequencies of anti-Rh immunization following AP-PC and BC-PC.

## Patients and Methods

As one of the largest tertiary and university hospitals of Switzerland (Inselspital, University Hospital, Insel Group, Bern, Switzerland), we have transfused 18,033 RBC, 7,020 plasma, and 4,371 platelet units in 2016. RBC and platelet products have been provided by the Interregional Blood Transfusion Service Bern, Swiss Red Cross (Bern, Switzerland) (IRB) and the plasma units (OctaplasLG<sup>®</sup>) by Octapharma (Lachen, Switzerland). In agreement with our national recommendations, we consider BC-PC and AP-PC clinically equivalent, except for HLA- and/or HPA-alloimmunized patients, who mandatorily receive 'best procurable' compatible single donor AP-PC. According to internal standards, a valid serological antibody screen (i.e., performed within the last 96 h) has been mandatory for all patients before any RBC and PC transfusion. For antibody screening and additional antibody identification (indirect antiglobulin and enzyme test), we used BioRad column agglutination technology (BioRad, Cressier, Switzerland). We performed all tests in compliance with the specific requirements of our national guidelines (<https://sbsc-bsd.ch/dokuman2/de-de/bsd/vorschriften/empfehlungen.aspx>).

Between August 2015 and September 2017, we found 5,799 positive antibody screening tests in 89,190 immunohematologically analyzed patients. We prospectively registered all newly detected Rh alloantibodies (D and non-D), which could not be explained by incompatible RBC transfusions. For every patient with a newly detected Rh alloantibody we compiled the complete transfusion history. According to antibody specificity, we identified all incompatible PC and/or RBC transfusions. Only patients with newly detected Rh alloantibodies who had not received any cognate Rh antigen-incompatible RBC transfusion but had been exposed to at least one incompatible PC were included in our study.

Data analyzed of the patients identified to have obviously been alloimmunized through PC transfusions included: age, gender, ABO blood group and Rh phenotype, main diagnoses, immunosuppressive drugs, transfusion history (Inselspital only), and date of alloantibody detection [7]. Transfusion history included every RBC and PC unit transfusion.

Recorded data of donors/PC/transfusions included: gender, age, ABO blood group and Rh phenotype, AP-PC or BC-PC, and date of transfusion. Molecular screening for confirmation of serologically Rh-negative donors is mandatory in Switzerland since 2015 [26].

We performed routine quality control for residual RBC count for 10 AP-PC and 10 BP-PC every month using the manual/visual cell counting chamber

method (Neubauer Chamber). We calculated geometric mean and range including all quality control RBC counts for AP-PC and BC-PC from January 2016 to December 2016.

Based on previous publications, we considered newly detected antibodies following a Rh-incompatible PC transfusion until day 27 as secondary immune response (booster effect) and those found on day 28 or later as a primary immune response against cognate Rh antigens [7, 8].

## Results

### *Detection of Newly Acquired Rh Antibodies after Platelet Transfusion*

From August 2015 to September 2017 a total of 4,596 AP-PC and 5,050 BC-PC were transfused to patients at our institution. In 11 patients (table 1) we identified 13 newly acquired Rh antibodies, which were triggered by a PC transfusion (table 2). Of the newly detected antibodies, 6 were of the specificity anti-D (patients #2, #4, #5, #6, #8, #10). For patients #2 and #6, a booster effect has to be assumed, considering the interval between the last Rh phenotype-incompatible transfusion and antibody detection. For patient #5, we interpreted rather a primary immunization because of weak reactivity in the antibody screen and identification tests. Anti-E alloantibodies were detected in 4 cases (patients #3, #7, #9, #11 (booster effect)), anti-c in 2 cases (patients #3, #7), and anti-f in 1 case (patient #1 (most likely also a booster effect)) (details in table 2). These 11 patients were investigated in detail.

### *Demographic Data, Underlying Diseases, and Therapy Resulting in Reduced Immune Function*

There were 6 males and 5 females with a median age of 67 years (range 53–80 years). Causes of hospitalization were hematological malignancies in 6 cases: Four patients had acute myeloid leukemia, one of them acute promyelocytic leukemia. One patient each had T-angioimmunoblastic leukemia or multiple myeloma. In 1 patient, we documented thrombocytopenia of unclear origin. Solid tumors were documented in 2 patients. Two patients had major heart surgery.

Patients #1, #3, #4, #7, #9, and #11 received cytotoxic chemotherapy; patient #8 received all-trans-retinoic acid and arsenic trioxide; patient #10 received demethylating agents. Therefore, 8 out of 11 patients have received treatments that may compromise immune function.

### *Transfusion History*

The 11 patients received 156 PC (83 BC-PC; 73 AP-PC). Patient #1 received a single AP-PC, whereas 3 patients (#2, #4, and #6) received BC-PC only. The other 7 patients (#3, #5, #7, #8, #9, #10, and #11) received both types of PC.

Ten of the 11 patients received RBC transfusions in addition (total number 152, range 3–41). All RBC units were antigen-negative for the identified alloantibodies and therefore excluded as cause for the alloimmunization.

Because of the known latency of up to 6 months for alloantibody appearance after antigen exposure [27], definite conclusions

about the incompatible PC of a series, which may have been the responsible trigger for the individual alloimmunization is not possible. We nonetheless documented the type (BC-PC or AP-PC unit), the number and the time point of the first incompatible transfusion in every patient's transfusion history (details summarized in table 2).

The patients received a total of 75 incompatible BC-PC and 31 incompatible AP-PC (table 2). The first incompatible transfusion was in 4 cases an AP-PC and in 9 cases a BC-PC. Five patients (#2, #4, #5, #6, and #11) received incompatible BC-PC only, whereof 3 patients received 1 unit each, and 2 patients received 5 units each. Patient #1 received incompatible AP-PC only (1 unit), whereas 5 patients comprising #3 and #7 (with respect to both allo-antibodies), #8, #9, and #10 received incompatible BC-PC as well as AP-PC. Details are summarized in table 2.

Three of the 11 patients (#6, #8, and #11) already had a known alloantibody and therefore were known to be at a higher risk for additional antibody formation after subsequent transfusions [28–32].

### *Contamination of PC by RBC*

Monitoring the RBC contamination is a mandatory element of the regular monthly quality controls of PC. There have been no changes of production processes during the study period. The results of the year 2016 showed a mean contamination of 0.304 (range 0.152–1.662) and 0.014 (range 0.003–0.080)  $\times 10^9$  RBC/l for BC-PC and AP-PC, respectively (table 3). Thus, the level of RBC contamination was more than tenfold higher in BC-PC than in AP-PC.

## Discussion

As a part of hemovigilance all newly detected alloantibodies have to be reported to the competent authority (Swissmedic). The work-up of such an alloantibody in 2015 revealed an anti-f specificity. Because of the appearance within 3 days, a booster effect seemed reasonable. However, the appearance of the alloantibody surprisingly was induced through an AP-PC transfusion. This prompted us to a prospective assessment of every single newly identified alloantibody with respect to the putative responsible transfused blood unit. Between August 2015 and September 2017, we detected 13 Rh antibodies in 11 patients, which must have been triggered by residual antigen-positive RBC of PC transfusions. Particularly, the spectrum and frequency of non-D Rh antibodies triggered by PC transfusions has exceeded our prospects.

So far, only few publications reported about formation of non-D Rh antibodies after PC transfusion. Kitazawa and colleagues [24] observed 3 patients who developed 4 alloantibodies (2 anti-E, 1 anti-C, and 1 anti-c) after platelet transfusions. These patients only received AP-PC. Because RBC contamination in AP-PC (range 0.00017–0.009 ml RBC per PC) [8], is probably insufficient to trigger alloimmunization (according to literature the minimum is around 0.03–0.05 ml RBC) [8, 24, 25, 33], the authors hypothe-

**Table 2.** Transfusion history of the 11 patients, in whom platelet transfusion resulted in 13 newly detectable Rh antibodies

Patient	Known antibody	New antibody	Date of detection	Days until detection	First incompatible transfusion	Type of first incompatible transfusion	Last incompatible transfusion before detection	Date of last negative type and screen test	Negative type & screens between first incompatible transfusion and detection	Negative type & screens before first incompatible transfusion	Antigen+/total units		
											RBC	BC-PC	AP-PC
1	none	anti-f*	24.08.15	3	21.08.15	AP-PC	n.a.	n.a.	n.a.	n.a.	0/13	0	1/1 (f-pos)
2	none	anti-D* <sup>1</sup>	08.10.15	28	10.09.15	BC-PC	10.09.15 (BC)	09.09.15	0	2	0/3	1/1 (D-pos)	0
3	none	anti-c/-E	10.11.15	40	01.10.15	BC-PC	19.10.15 (BC, c+)	02.11.15	7 (c-neg)	3 (c-neg)	0/12	10/10 (c-pos)	5/7 (c-pos)
				36	05.10.15	BC-PC	19.10.15 (BC, E+)		6 (E-neg)	4 (E-neg)		7/10 (E-pos)	1/7 (E-pos)
4	none	anti-D	15.12.15	109	28.08.15	BC-PC	28.08.15 (BC)	01.09.15	1	2	0/8	1/1 (D-pos)	0
5	none	anti-D <sup>2</sup>	22.05.16	25	27.04.16	BC-PC	27.04.16 (BC)	12.05.16	2	1	0/8	1/1 (D-pos)	0/1 (D-pos)
6	anti-K & anti-Co(b)	anti-D*	03.06.16	6	28.05.16	BC-PC	30.05.16 (BC)	28.05.16	0	1	0	5/6 (D-pos)	0
7	none	anti-c/-E	03.06.16	1414	19.07.12	AP-PC (c-pos)	31.05.16 (c-pos)	27.05.16	26	1	0/41	24/24 (c-pos)	13/19 (c-pos)
					19.07.12	AP-PC (E-pos)	31.05.16 (c-pos)					17/24 (E-pos)	5/19 (E-pos) <sup>3</sup>
8	anti-E	anti-D	23.08.16	41	13.07.16	BC-PC	04.08.16 (BC)	20.08.16	8	1	0/11	2/2 (D-pos)	1/10 (D-pos)
9	none	anti-E	19.11.16	57	23.09.16	BC-PC	17.11.16 (BC)	15.11.16	12	2	0/25	18/25 (E-pos)	6/21 (E-pos)
10	none	anti-D	03.08.17	46	18.06.17	AP-PC	02.08.17 (BC)	30.07.17	18	5	0/24	8/8 (D-pos)	5/9 (D-pos)
11	anti-K & anti-Kp(a)	anti-E*	02.09.17	13	20.08.17	BC-PC	28.08.17 (BC)	25.08.17	1	3	0/7	5/5 (E-pos)	0/4 (E-pos)

n.a = Data not available/applicable.

\*Probably booster-effect of previously immunized patient.

<sup>1</sup>Day 28 post transfusion, but 4+ reactivity.

<sup>2</sup>Day 25 post transfusion, but weak reactivity.

<sup>3</sup>Rh phenotype from one donor is missing.

**Table 3.** Contamination of the PC by RBC (quality controls of 2016)

PC	Mean (range) RBC × 10 <sup>9</sup> /l	Equates to an estimated mean blood volume, ml
AP-PC	0.014 (0.003–0.080)	0.0028
BC-PC	0.304 (0.152–1.662)	0.0608

sized that RBC microparticles (being present in BC-AP as well as AP-PC) could contribute to alloimmunization. Microparticles can be phagocytosed easily and so are probably more immunogenic than RBCs [34, 35].

In our study we suggest that AP-PC triggers a booster effect of an anti-f alloantibody (#1), and we therefore suppose that the minimum amount of RBC (and probably also of microparticles) as a trigger of alloimmunization should be critically discussed [24].

According to our quality controls, RBC contamination was higher in BC-PC than in AP-PC, which was in line with the literature and supports that an increasing antigen dose enhances the potential for immunization [33]. For appraisal of the impact of transfusing either AP-PC or BC-PC (representing different amounts of RBC contamination) on the risk of Rh alloimmunization, we analyzed in more detail the Rh-incompatible PC transfused. Altogether, the 11 patients described in our investigation received 156 PC (83 BC-PC and 73 AP-PC). The Rh-alloimmunized patients received more incompatible BC-PC (75/83) than AP-PC (31/73). In the majority of cases, the first incompatible transfusion was a BC-PC (9 out of 13). Finally, 5/11 patients had received incompatible BC-PC only. Due to our limited set of data and the heterogeneity of our small study population, it is not possible to make a reliable assessment about the different alloimmunization risk of either group (BC-PC vs. AP-PC). However, the risk for alloimmunization after BC-PC seems to be higher than after AP-PCs, as supported by previous reports of others [7, 33].

More recently, Schonewille and colleagues [27] reported on the incidence of alloantibody formation after ABO/RhD or extended matched RBC transfusions. In their randomized trial in surgical patients (MATCH study), they tried to avoid clinically relevant RBC antibody formation by extended (c, C, E, K, Fya, Jka, and S antigens) matched RBC transfusions. Among the group of patients who received PC (reported to be BC-PC only) in addition to RBCs transfusions, they surprisingly found 6 patients who have formed 8 RBC alloantibodies (5× anti-E, 1× each anti-D, anti-K, and anti-e) after cognate RBC antigen exposure through platelet transfusions only. Moreover, a significant absolute risk difference in favor of extended RBC matching was only shown in the group of patients who did not receive PC transfusions. The authors concluded that RBC-extended matching is only successful if the patient does not receive accompanying non-RBC antigen-matched platelet products [27]. The results of our study with regard to spectrum and frequency of non-D Rh antibodies triggered by platelet transfusion are in line with these reports and strongly support the significant potential of PC transfusions for inducing RBC alloantibodies.

The rate of RhD alloimmunization is known to depend not only on the RBC content of the PC but also on a variety of further

factors, including the immune status of the patient and the rate of secondary immune response [33]. Age (median, 67 years) and gender distribution (f/m = 5/6) of our patients was within expected ranges in the hospital. A high proportion of the patients had either hematological malignancies or solid tumors. They received cytotoxic chemotherapy or other anti-leukemic treatments such as ATRA/ATO or demethylating compounds. The remaining patients had either idiopathic thrombocytopenia or heart surgery (further details in table 1). Our results are in line with the reports of others that Rh alloimmunization through PC is observed in immunocompetent as well as immunocompromised patients [7, 33]. The newly detected alloantibodies in our series were most frequently anti-D (6/13), followed by anti-E (4/13), anti-c (2/13), and anti-f (1/13), details in table 2. In 4 patients (2 with anti-D, 1 with anti-E, 1 with anti-f), a booster effect has to be assumed. In patient #7, there was a gap of 4 years between the last negative type and screen testing and the detection of the alloantibody at our institute. Therefore, an alternative immunization event might have taken place during that period of time.

Our observation underlines the potential of residual RBC in PC for primary immunization or boosting effect against RBC antigens. A secondary immune response is known to arise as a ‘boosting’ effect after much smaller antigenic provocation than that of the primary response [27, 33]. In our study population, we can describe a primary or secondary immune response to PC transfusions in 10 out of 11 patients (for #7 an alternative immunization event cannot be ruled out). In 8 of these 10 patients, a primary immunization can be assumed and in 2/10 patients (#1 and #6) a secondary immunization seems to be more likely. Regarding the latter 2 patients, reasons for primary immunization are likely to be from previous transfusions (#1 and #6) and/or pregnancies (#6), but no specific data are available to prove the reason for primary immunization.

In conclusion, BC-PC and AP-PC transfusions may not only induce RhD alloimmunization, but also immunization against further Rh antigens such as c, E, and f. According to the current literature, the risk seems higher for BC-PC than for AP-PC. Interestingly, in 1 case we found a booster effect of an anti-f alloantibody triggered by one single AP-PC. Therefore, the minimum amount of RBC as a trigger of alloimmunization (booster effect or primary antibody formation) may be reassessed. Being aware of problems with respect to feasibility, these results may have impact on the next edition of our national guidelines. The responsible national working party will discuss a modification of their recommendations according to PC transfusion with respect to Rh and Kell compatibility (e.g. recommendation of AP-PC with extended Rh phenotype and Kell matching for selected patient populations such as females of childbearing age or chronically transfused hemoglobinopathy patients). In addition, remarks about immunologic safety of upper limits of RBC contamination in PC as well as clinical equivalence of BC-AP and AP-PC with respect to immunogenicity will be reconsidered. Our limited set of data does not allow the calculation of a relative risk for alloimmunization through PC. In order to answer this question, larger prospective studies are needed.

## Disclosure Statement

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

## References

- 1 Simon TL, McCullough J, Snyder EL, Solheim BG, Strauss RG (eds): *Rossi's Principles of Transfusion Medicine*, 5th ed. Chichester, Wiley-Blackwell, 2016.
- 2 Kaiser-Guignard J, Canellini G, Lion N, Abonnenc M, Osselaer JC, Tissot JD: The clinical and biological impact of new pathogen inactivation technologies on platelet concentrates. *Blood Rev* 2014;28:235–241.
- 3 Goldfinger D, McGinniss MH: Rh-incompatible platelet transfusions – risks and consequences of sensitizing immunosuppressed patients. *N Engl J Med* 1971;284:942944.
- 4 ScheultJN, Triulzi DJ, Yazer MH: I am the 9%: making the case for whole-blood platelets. *Transfus Med* 2016; 26:177–185.
- 5 Cid J, Lozano M: Risk of Rh(D) alloimmunization after transfusion of platelets from D+ donors to D- recipients. *Transfusion* 2005;5:453; author reply 453–454.
- 6 Cid J, Carbassé G, Pereira A, Sanz C, Mazzara R, Escolar G, Lozano M: Platelet transfusions from D+ donors to D- patients: a 10-year follow-up study of 1014 patients. *Transfusion* 2011;51:1163–1169.
- 7 Cid J, Lozano M, Ziman A, West KA, O'Brien KL, Murphy MF, Wendel S, Vázquez A, Ortín X, Hervig TA, Delaney M, Flegel WA, Yazer MH; Biomedical Excellence for Safer Transfusion collaborative: Low frequency of anti-D alloimmunization following D+ platelet transfusion: the Anti-D Alloimmunization after D-incompatible Platelet Transfusions (ADAPT) study. *Br J Haematol* 2015;168:598–603.
- 8 Cid J, Yaze MH, Lozano M: Platelet transfusion and respecting patient D type. *Curr Opin Hematol* 2015;22: 540–546.
- 9 Pollack W, Ascari WQ, Crispen JF, O'Connor RR, Ho TY: Studies on Rh prophylaxis. II. Rh immune prophylaxis after transfusion with Rh-positive blood. *Transfusion* 1971;11:340–344.
- 10 Urbaniak SJ, Robertson AE: A successful program of immunizing Rh-negative male volunteers for anti-D production using frozen/thawed blood. *Transfusion* 1981;21:64–69.
- 11 Anderson KC, Ness PM: *Scientific Basis of Transfusion Medicine: Implications for Clinical Practice*. Philadelphia, W.B. Saunders, 2000.
- 12 Lozano M, Cid J: The clinical implications of platelet transfusions associated with ABO or Rh(D) incompatibility. *Transfus Med Rev* 2003;17:57–68.
- 13 Urbaniak SJ: Alloimmunity to RhD in humans. *Transfus Clin Biol* 2006;13:19–22.
- 14 Lichtiger B, Surgeon J, Rhorer S: Rh-incompatible platelet transfusion therapy in cancer patients. A study of 30 cases. *Vox Sang* 1983;45:139–143.
- 15 Baldwin ML, Ness PM, Scott D, Braine H, Kickler TS: Alloimmunization to D antigen and HLA in D-negative immunosuppressed oncology patients. *Transfusion* 1988;28:330–333.
- 16 McLeod BC, Piehl MR, Sasseti RJ: Alloimmunization to RhD by platelet transfusions in autologous bone marrow transplant recipients. *Vox Sang* 1990;59:185–189.
- 17 Heim MU, Böck M, Kolb HJ, Schleuning M, Mempel W: Intravenous anti-D gammaglobulin for the prevention of rhesus isoimmunization caused by platelet transfusions in patients with malignant diseases. *Vox Sang* 1992;62:165–168.
- 18 Zeiler T, Wittmann G, Zingsem J, Weisbach V, Zimmermann R, Eckstein R: A dose of 100 IU intravenous anti-D gammaglobulin is effective for the prevention of RhD immunisation after RhD-incompatible single donor platelet transfusion. *Vox Sang* 1994;66:243.
- 19 Moncharmont P, Barday G, Meyer F: Red blood cell alloimmunisation after platelet transfusion: a 5-year study. *Blood Transfus* 2014;12(suppl 1):s147–148.
- 20 O'Brien KL, Haspel RL, Uhl L: Anti-D alloimmunization after D-incompatible platelet transfusions: a 14-year single-institution retrospective review. *Transfusion* 2014;54:650–654.
- 21 Dunbar NM, Katus MC, Freeman CM, Szczepiorowski ZM: Easier said than done: ABO compatibility and D matching in apheresis platelet transfusions. *Transfusion* 2015;55:1882–1888.
- 22 Weinstein R, Simard A4, Ferschke J, Vauthrin M, Bailey JA, Greene M: Prospective surveillance of D- recipients of D+ apheresis platelets: alloimmunization against D is not detected. *Transfusion* 2015;55:1327–1330.
- 23 Cid J, Lozano M, Klein HG, Flegel WA: Matching for the D antigen in haematopoietic progenitor cell transplantation: definition and clinical outcomes. *Blood Transfus* 2014;12:301–306.
- 24 Kitazawa J, Nolle K, Morioka H, Tanaka K, Inomata M, Kubuki Y, Ohto H: Non-D Rh antibodies appearing after apheresis platelet transfusion: stimulation by red cells or microparticles? *Vox Sang* 2011;100:395–400.
- 25 Klein HG, Anstee DJ (eds): *Mollison's Blood Transfusion in Clinical Medicine*. Chichester, Wiley & Sons, 2005.
- 26 Crottet SL, Henny C, Meyer S, Still F, Stolz M, Gottschalk J, Neuenschwander K, Taleghani BM, Gowland P, Frey BM, Fontana S, Hustinx H, Niederhauser C, Gassner C: Implementation of a mandatory donor RHD screening in Switzerland. *Transfus Apher Sci* 2014;50:169–174.
- 27 Schonewille H, Honohan Á, van der Watering LM, Hudig F, Te Boekhorst PA, Koopman-van Gemert AW, Brand A: Incidence of alloantibody formation after ABO-D or extended matched red blood cell transfusions: a randomized trial (MATCH study). *Transfusion* 2016;56:311–320.
- 28 Tormey CA, Fisk J, Stack G: Red blood cell alloantibody frequency, specificity, and properties in a population of male military veterans. *Transfusion* 2008;48: 2069–2076.
- 29 van de Watering L, Hermans J, Witvliet M, Versteegh M, Brand A: HLA and RBC immunization after filtered and buffy coat-depleted blood transfusion in cardiac surgery: a randomized controlled trial. *Transfusion* 2003;43:765–771.
- 30 Schonewille H, van de Watering LM, Brand A: Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? *Transfusion* 2006;46:630–635.
- 31 Schonewille H, Klumper FJ, van de Watering LM, Kanhai HH, Brand A: High additional maternal red cell alloimmunization after Rhesus- and K-matched intrauterine intravascular transfusions for hemolytic disease of the fetus. *Am J Obstet Gynecol* 2007;196: 143.e1–6.
- 32 Schonewille H, de Vries RR, Brand A: Alloimmune response after additional red blood cell antigen challenge in immunized haematooncology patients. *Transfusion* 2009;49:453–457.
- 33 Hendrickson JE, Eisenbarth SC, Tormey CA: Red blood cell alloimmunization: new findings at the bench and new recommendations for the bedside. *Curr Opin Hematol* 2016;23:543–549.
- 34 Kumpel B: Are weak D RBCs really immunogenic? *Transfusion* 2006;46:1061–1062; discussion 1062–1066.
- 35 Canellini G, Rubin O, Delobel J, Crettaz D, Lion N, Tissot JD: Red blood cell microparticles and blood group antigens: an analysis by flow cytometry. *Blood Transfus* 2012;10(suppl 2):s39–45.