

Original article

Comparison of ABO antibody titers in apheresis platelet donors and single donor platelets in a platelet additive solution

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Abstract:

Introduction: Single donor platelets (SDPs) contain approximately 300 mL of plasma. ABO antibodies in SDP may cause hemolysis if transfused to an ABO-incompatible recipient. Currently, one-third of the plasma in SDP is replaced with platelet additive solution (PAS) to lower ABO antibodies. **Objective:** We aimed to compare the ABO antibody titers in apheresis platelet donors and SDP-PAS. **Materials and Methods:** Sixty platelet donors, 20 each from groups O, A, and B, were enrolled. Anti-A and anti-B titers were measured using the automated column agglutination technique (CAT) at room temperature (RT) and anti-human globulin phases (AHG). **Results:** All 60 participants were male, aged 23-63 years. The anti-A titers in donors ranged from 2 to 2,048, while the anti-B titers ranged from 4 to 8,192. The titer > 256 was observed only in group O donors. The anti-A and anti-B titers in SDP-PAS ranged from 2-512 and 8-2,048, respectively. The ABO titers in SDP-PAS were ≤ 64 in 100% of groups A and B, and in 60% of group O. Compared to the titers in donors, the titers of SDP-PAS were significantly reduced by two titers on average ($p < 0.001$). **Conclusion:** There were group O platelet donors with an exceptionally high titer, all SDP-PAS groups A and B had a titer of 64 or lower. Testing of ABO antibody titers in group O SDP-PAS is recommended to ensure transfusion safety.

Keywords : ● Single donor platelets (SDPs) ● ABO antibody titers ● Column agglutination technique (CAT) ● Platelet additive solution (PAS)

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นิพนธ์ต้นฉบับ

การศึกษาเปรียบเทียบระดับเอบีโอแอนติบอดีในผู้บริจาคเกล็ดเลือดและเกล็ดเลือดจากผู้บริจาครายเดียวที่ผสมน้ำยาเลี้ยงเกล็ดเลือด

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บทคัดย่อ

บทนำ เกล็ดเลือดของผู้บริจาครายเดียว (SDP) ประกอบด้วยพลาสมาประมาณ 300 มิลลิลิตร ABO แอนติบอดีในเกล็ดเลือดของผู้บริจาครายเดียว อาจทำให้เม็ดเลือดแดงแตกหากนำไปให้กับผู้รับที่หมู่เลือด ABO เข้ากันไม่ได้ ปัจจุบันพลาสมาปริมาณ 1 ใน 3 ของผู้บริจาครายเดียวถูกแทนที่ด้วยน้ำยาเลี้ยงเกล็ดเลือด (PAS) เพื่อลด ABO แอนติบอดี **วัตถุประสงค์** เพื่อเปรียบเทียบระดับ ABO แอนติบอดีในผู้บริจาคเกล็ดเลือด และเกล็ดเลือดจากผู้บริจาครายเดียวที่ผสมน้ำยาเลี้ยงเกล็ดเลือด (PAS) **วัสดุและวิธีการ** การศึกษานี้ทำในผู้บริจาคเกล็ดเลือดหมู่ O, A และ B จำนวนหมู่ละ 20 ราย โดยวัดค่า titers ของ anti-A และ anti-B ที่อุณหภูมิห้อง (room temperature; RT) และ anti-human globulin (AHG) phase โดยวิธี column agglutination technique โดยใช้เครื่องตรวจอัตโนมัติ **ผลการศึกษา** ผู้เข้าร่วมวิจัยทั้งหมด 60 คน เป็นชาย อายุตั้งแต่ 23-63 ปี anti-A titers ในผู้บริจาคเกล็ดเลือดอยู่ระหว่าง 2 ถึง 2,048 ในขณะที่ anti-B titers อยู่ระหว่าง 4 ถึง 8,192 ค่า titer > 256 พบเฉพาะในผู้บริจาคเกล็ดเลือดหมู่ O anti-A titers และ anti-B titers ใน SDP-PAS มีค่าตั้งแต่ 2-512 และ 8-2,048 ตามลำดับ สำหรับ SDP-PAS หมู่ A และ B 100% มีค่า ABO titer ≤ 64 แต่ในหมู่ O มีเพียง 60% เมื่อเทียบกับผู้บริจาคเกล็ดเลือด เอบีโอแอนติบอดีมีการลดลงโดยเฉลี่ย 2 titers และลดลงอย่างมีนัยสำคัญ (p < 0.001) **สรุป** ผู้บริจาคเกล็ดเลือดหมู่ O บางรายมีระดับ titer สูงมาก และ SDP-PAS หมู่ A และ B ทั้งหมดมีระดับ แอนติบอดี titer ≤ 64 แนะนำให้ทำการทดสอบ ABO แอนติบอดี titer ในหมู่ O SDP-PAS เพื่อความปลอดภัยในการให้เกล็ดเลือด

คำสำคัญ : ● เกล็ดเลือดจากผู้บริจาครายเดียว ● ความแรงของแอนติเอและแอนติบี ● วิธีคอลัมน์แอกกลูตินเนชั่น
● น้ำยาเลี้ยงเกล็ดเลือด

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2567;34:19-26.

Introduction

ABO is the most important blood group system in clinical transfusion because of the presence of antigens on red blood cells and naturally occurring corresponding antibodies in serum or plasma.¹⁻³ Platelets are transfused to treat bleeding or used prophylactically to prevent bleeding in thrombocytopenic patient.⁴ International guidelines recommend the transfusion of ABO-compatible platelets to achieve a good increment. However, when ABO type-specific platelets are not available, transfusion of different ABO types is an acceptable practice.⁵⁻⁶ Transfusion of ABO-incompatible platelets leads to passive transfer of anti-A and/or anti-B antibodies, which may cause acute hemolytic transfusion reactions and/or a positive direct antiglobulin test.⁷ Therefore, when ABO-incompatible platelet transfusion is unavoidable, it is recommended to select whole blood-derived platelets that came from several donors to avoid the chance of transfusion of a high-titer ABO antibody from only one donor. Because it is believed that the hemolytic transfusion reaction is related to the high ABO antibody titer.⁸ However, on many occasions, whole blood-derived platelets are unavailable. The single donor platelets have to be issued for an ABO-incompatible transfusion instead. Consequently, many blood banks must prepare their single donor platelets as safely as possible for ABO-incompatible transfusions.

Several approaches exist to reduce the risk of adverse reactions related to ABO-incompatible platelet transfusions, such as reduction of plasma volume, washing the platelets, screening for low antibody titer donors, or use of platelets in platelet additive solutions (PAS).⁹⁻¹⁰ The PAS replaces about 20-80% of the plasma volume in single donor platelets (SDPs), so the titer of isoagglutinin should be lessened.⁹ However, it is unknown how much the titer has diminished. Moreover, whether the titer reduction is enough for safe ABO-incompatible platelet transfusion is still in doubt, especially for the products derived from high antibody titer donors. Therefore, determining the final antibody titer of single

donor platelets in PAS and looking for the relationship between the ABO antibodies titers in donors and those in the SDP-PAS may be useful to guide the preparation of platelets that are safe for ABO-incompatible platelet transfusion.

Nowadays, automatic analyzers are developed for immunohematology tests using the column agglutination technique (CAT), which provides a standardized test procedure to achieve reliable results. They can be used to determine antibody titration using a validated, standardized protocol. Fully automated systems can reduce errors in human operations and reading and result in accurate results, and reduce staff hand-on time.¹¹⁻¹³

Materials and Methods

This study was approved by the Siriraj Institutional Review Board under number 627/2022. Sixty apheresis platelet donors (20 donors of each blood group O, A, and B) were enrolled from September 7, 2022, to November 7, 2022. All apheresis platelet donors were qualified as guided by our national standards of blood banks and transfusion service.¹⁴ Apheresis platelet collection to achieve single donor platelets in platelet additive solution was done using a Trima Accel blood cell separator (Terumo BCT, Ltd., Lakewood, CO, USA) using an automated blood collection system tubing set (Terumo BCT, Ltd., Lakewood, CO, USA) according to manufacturer protocol. We used platelet additive solution (T-PAS, Terumo BCT, Ltd., Lakewood, CO, USA) to achieve the ratio of plasma to platelet additive solution of 35:65.

EDTA blood samples were collected from the diversion pouch bag of the apheresis platelet bag. The samples from the SDP-PAS were collected from the final product bag. The titers of anti-A and anti-B antibodies were tested at both room temperature (RT) and anti-human globulin test (AHG) phases using CAT on a fully automated system (IH-500 Bio-Rad Laboratories Ltd., Cressier, Switzerland) according to the manufacturer's protocol. For room temperature (RT) anti-A and anti-B titers, we used 0.8% A1 and B cells (ID-Diacell ABO,

Bio-Rad Laboratories Ltd., Cressier, Switzerland) and the neutral cards (ID Card, Bio-Rad). The anti-human globulin test (AHG) was tested on the LISS/Coombs card (anti-IgG, C3d; polyspecific cassette, Bio-Rad). All tested procedures used an endpoint reaction of 1+. In the case of samples that reached maximum titer by the automated system, we further diluted the patient samples and tested them manually with CAT using the same reagents to determine the final titers.

Statistical analysis

The age distribution of apheresis platelet donors was assessed using descriptive statistical analysis. The Wilcoxon signed-rank test was used to compare anti-A and/or anti-B titers in apheresis platelet donors and single donor platelets in a platelet additive solution. The *p*-values of less than 0.05 were considered significant. The software SPSS 21.0 was used for statistical analysis.

Results

All donors were male with a mean age of 46.06 (range 23-63) years old. The mean ages of the apheresis platelet donor groups (O, A, and B) donors were 46.10±11.06, 45.65±8.69, and 44.45±11.10 years, respectively. There was no statistical difference in age between blood donors in the three different groups (groups O vs. A, group O vs. B, and group A vs. B: *p* = 0.800, 0.143, and 0.920).

The distribution of anti-A and anti-B titers in apheresis platelet donor groups O, A, and B that reacted at RT and AHG, as determined by CAT, is shown in Figure 1. The results showed that AHG anti-A titers of groups O and B were significantly higher than RT titers (*p* = 0.003 and *p* = 0.001). In contrast, anti-B titers at AHG phase of groups O and A were not significantly different (*p* = 0.167 and *p* = 0.314).

ABO antibody titers in SDP-PAS

The distribution of anti-A and anti-B titers in SDP-PAS of groups O, A, and B that reacted at RT and AHG by CAT is shown in Figure 2. The results showed that the anti-A titers of groups O and B reacted at the AHG phase were higher than the RT titer (*p* = 0.001 and *p* =

0.001). Anti-B titers of SDP-PAS groups O and A at AHG were not significantly different (*p* = 0.712 and 0.301).

ABO antibody titers in SDP-PAS were significantly reduced compared to the titers in blood donors. Twelve of the 20 group O SDP-PAS (60%) and all groups A and B SDP-PAS had ABO titers ≤ 64. In eight groups O SDP-PAS with ABO antibodies ≥ 128, five units had high AHG anti-B titers (titers 128, 128, 128, 512, and 2,048), two units had high AHG anti-A titers of 128 and the last unit had RT and AHG anti-A titer 128 and 512, respectively.

Comparison of ABO antibody titers determined by CAT in apheresis platelet donors and single donor platelet in PAS (SDP-PAS)

Both anti-A and anti-B titers reacted at RT and AHG in the SDP-PAS, which showed a significant decrease compared to those in apheresis platelet donors (*p* < 0.001). A comparison of ABO antibody titers in blood donors and SDP-PAS in blood groups O, A, and B by CAT is shown in Table 1. The reduction of ABO antibody titers in SDP-PAS compared with that in the donors was about twofold.

Discussion

This study demonstrated the ABO antibody titer in apheresis platelet donors compared with single donor platelets in platelet additive solution in the same donor by measuring anti-A and anti-B titers by CAT. The result showed that individual apheresis platelet donors had different levels of antibody titers. Some apheresis platelet donors had very high antibody titers, as found in group O apheresis platelet donors with an anti-B titer of 8,192, while in SDP-PAS collected from the same donor, the anti-B titer was 2,048. Therefore, we have retested antibody titers in the same apheresis platelet donor, which still have a high titer of 8,192. However, SDP-PAS in blood groups O, A, and B had a significant reduction of the anti-A titers and anti-B titers compared to apheresis platelet donors.

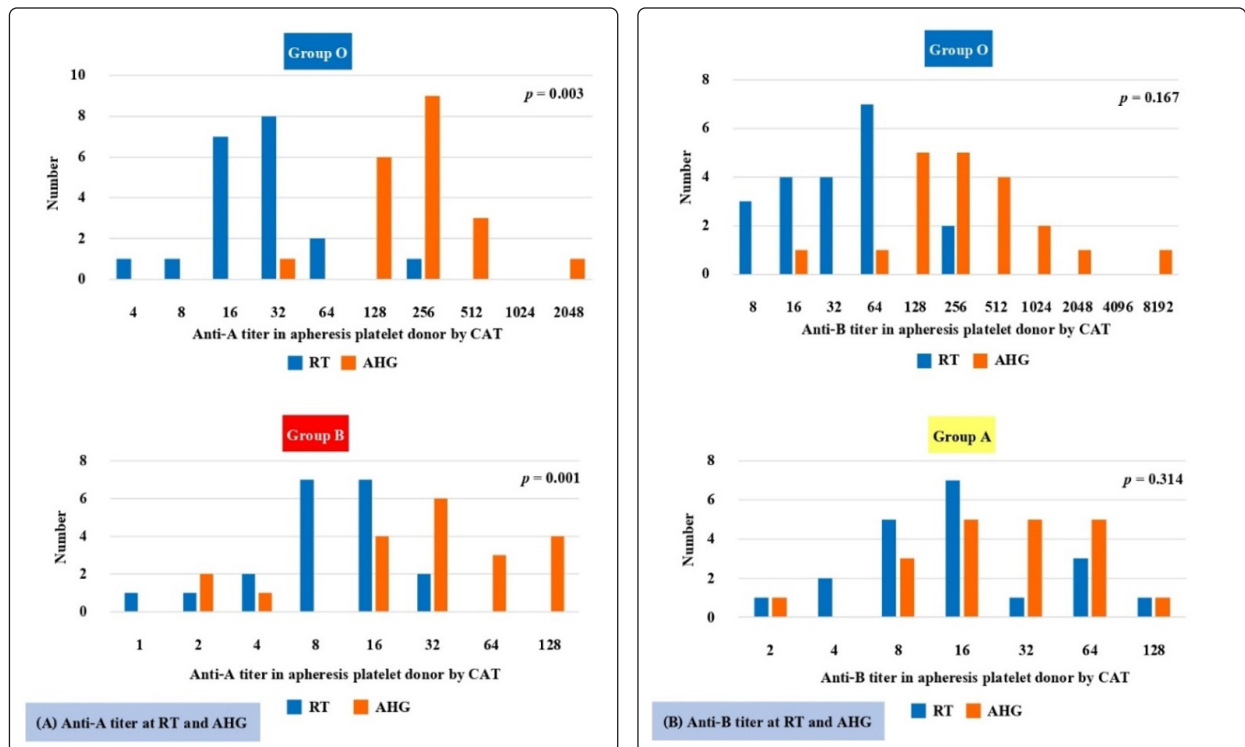


Figure 1 Distribution of the ABO antibody titers in apheresis platelet donors determined by CAT

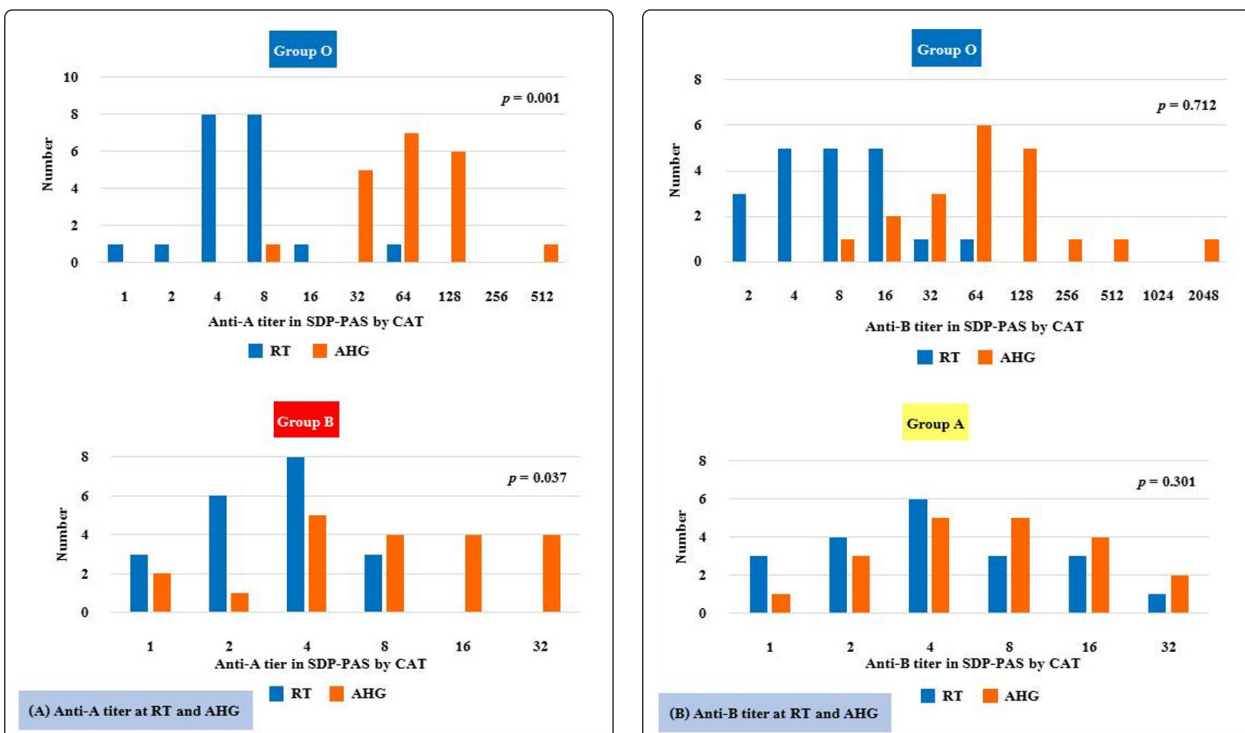


Figure 2 Distribution of the ABO antibody titers in SDP-PAS determined by CAT

Table 1 Comparison of ABO antibody titers between apheresis platelet donors and SDP-PAS in blood groups O, A, and B by CAT

Blood group antibody	Donor / platelet	Antibody titers at			
		RT phase		AHG phase	
		Median (IQR)	Range	Median (IQR)	Range
O, anti-A	Donor	32 (16,32)	4-256	256 (128,256)	32-2048
	SDP-PAS	8 (4,8)	1-64	64 (32,128)	8-128
B, anti-A	Donor	8 (8,16)	1-32	32 (16,64)	2-128
	SDP-PAS	4 (2,4)	1-8	8 (4,16)	1-32
O, anti-B	Donor	32 (16,64)	8-256	256 (128,512)	16-8192
	SDP-PAS	8 (4,16)	2-64	64 (32,128)	8-2048
A, anti-B	Donor	16 (8,64)	2-128	32 (16,64)	2-128
	SDP-PAS	4 (2,8)	1-32	8 (4,16)	1-32

AHG = antihuman globulin; IQR = interquartile range; RT = room temperature; SDP-PAS = single donor platelets with platelet additive solution

There were studies about ABO antibodies in Thai blood donors by Mavichak *et al.* that reported the anti-B titer (4-1,024) to be slightly higher than the anti-A titer (4-512). The titer range of anti-A and anti-B was 4-512 and 4-1,024, respectively.¹⁵ Khampanon K, *et al.* reported that the IgM anti-A and anti-B titers in blood group O donors range from 8 to 1,024.¹⁶ They reported an ABO antibody titer slightly different than ours. Our study included only a small number of participants (n = 60) and we included all blood groups, not only group O. When analyzing only group O at RT, the ABO antibody titer is closer to the reported results. The other study from Gayathiri KC, *et al.* reported comparable titers for IgM only and reported an ABO antibody titer similar to our result. The median ABO antibody titers in apheresis platelets suspended in PAS were lower than in apheresis platelets suspended in plasma, similar to our results.¹⁷ Tendulkar AA, *et al.* studied anti-A and anti-B titers in group O platelet donors. They reported a high ABO antibody titer in group O platelet donors (anti-B titer of 2,048).¹⁸ When analyzing only group O, the anti-A titer and anti-B titer are closer to the report of our results.

On the other hand, Bazigou F, *et al.* studied group O apheresis donors by evaluating anti-A and anti-B titers. They reported that anti-A titers were in the range of

2-1,024 (median titer: 64), while anti-B titers were in the range of 2-256 (median titer: 32).¹⁹ When analyzing only group O apheresis donors, their results are different than ours.

Due to the fact that apheresis platelets are collected from only one donor, if the donors had high ABO titers, the platelet product may also have a high ABO antibody titer. With current technology which replaces part of the plasma in platelets with PAS, the ABO titers in SDP-PAS would not be as high. Our data confirmed the low titer ABO antibodies in all tested group A and group B SDP-PAS platelets, which ABO antibodies lower by about two fold from the donors' titer. However, we found one group O platelet donor who had an exceptionally high AHG anti-B titer of 8,192, and the SDP-PAS from this donor had an AHG anti-B titer of 2,048. Besides this, of the 20 group O SDP-PAS tested, 8 (40%) of the SDP-PAS had an ABO titer of 128 or higher.

There was no consensus about the critical ABO titers in platelet products.²⁰ But there were reported cases of hemolysis and fatalities after transfusion with high-titer single donor platelets from US²¹ before the era of using PAS; however, after using PAS, there was a report that high ABO antibody titers in plasma might lead to hemolysis in the recipient. The ABO antibody

titers that should be considered to have high risk for hemolytic reaction can be as low as 128 as found in reported cases from the US FDA.²¹ In other references, ABO RT titer exceed 100 and AHG titer exceed 400 were considered to have high risk for hemolytic reaction²² and from this criteria, our 3 units of SDP-PAS group O had high risk for hemolytic reaction too (one SDP-PAS had both RT anti-B titer of 128 and AHG anti-B 512, other two SDP-PAS had AHG anti-A titer 512 and 2,048). Therefore, we recommended that the SDP-PAS be tested for ABO antibody titers if the products will be transfused to ABO-incompatible recipients. Our results correlated with the other studies.²³

Conclusion

Antibodies in blood donors varied among individuals. We tested only a limited number of donors' blood, but we found unexpectedly high anti-B at AHG phases (titer of 8,192) in blood group O donors, while SDP-PAS from the same donor still had a high titer of 2,048. The ABO antibody titer in the SDP-PAS platelet product was significantly reduced from the titer in blood donors. All group A and B SDP-PAS ABO titers were < 64, however, the ABO titer in 40% of SDP-PAS group O were ≥ 128. We encourage blood centers to use PAS to reduce ABO antibody titers in single donor platelet products and measure ABO antibodies titer in group O SDP-PAS to identify the SDP-PAS unit with a high titer and avoid transfusing to an ABO incompatible recipient.

References

- Mitra R, Mishra N, Rath GP. Blood groups systems. *Indian J Anaesth.* 2014;58:524-28.
- Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen: *FactsBook*. 3rd ed. Amsterdam: Academic Press; 2012.
- Uevichitarun Y. The importance of detecting antibodies in the ABO system. *J Hematol Transfus Med.* 2015;25:347-54.
- Haematology BCfSi, Force BTT. Guidelines for the use of platelet transfusions. *Br J Haematol.* 2003;122:10-23.
- Malvik N, Leon J, Schlueter AJ, Wu C, Knudson CM. ABO-incompatible platelets are associated with increased transfusion reaction rates. *Transfusion.* 2020;60:285-93.
- Jain P, Tendulkar A, Gupta A. First Indian initiative for preparation of low-titer group "O" single-donor platelets with platelet additive solution. *Asian J Transfus Sci.* 2018;12:10-6.
- Karabin MS, Blagg L, Tobian AA, King KE, Ness PM, Savage WJ. ABO antibody titers are not predictive of hemolytic reactions due to plasma-incompatible platelet transfusions. *Transfusion.* 2012;52:2087-93.
- Quillen K, Sheldon SL, Daniel-Johnson JA, Lee-Stroka AH, Flegel WA. A practical strategy to reduce the risk of passive hemolysis by screening plateletpheresis donors for high-titer ABO antibodies. *Transfusion.* 2011;51:92-6.
- Mathur A, Swamy N, Thapa S, Chakraborty S, Jagannathan L. Adding to platelet safety and life: platelet additive solutions. *Asian J Transfus Sci.* 2018;12:136-40.
- van der Meer PF, Bontekoe IJ, Kruit G, Peeters G, van Toledo PJ, Tomson B, et al. Volume-reduced platelet concentrates: optimization of production and storage conditions. *Transfusion.* 2012;52:819-27.
- Park ES, Jo KI, Shin JW, Park R, Choi TY, Ban HI, et al. Comparison of total and IgG ABO antibody titers in healthy individuals by using tube and column agglutination techniques. *Ann Lab Med.* 2014;34:223-9.
- Bhangale A, Pathak A, Pawar S, Jeloka T. Comparison of antibody titers using conventional tube technique versus column agglutination technique in ABO blood group incompatible renal transplant. *Asian J Transfus Sci.* 2017;11:131-4.
- Matsuura H, Akatsuka Y, Matsuno T, Sugiura Y, Arakawa S, Oikawa S, et al. Comparison of the tube test and column agglutination techniques for anti-A/-B antibody titration in healthy individuals. *Vox Sang.* 2018;113:787-94.
- World Health Organization. Guidelines on assessing donor suitability for blood donation. *Library Cataloguing-in-Publication Data;* 2012.
- Mavichak W, Chiewsilp P, Tubrod J, Ovataga P. ABO antibodies among group O Thai blood donors. *J Hematol Transfus Med.* 2013;23:203-10.
- Khampanon K, Chanprakop T, Sriwanitchak P, Setthakarn M, Oota S, Nathalang O. The characteristics of ABO antibodies in group O Thai blood donors. *J Clin Lab Anal.* 2012;26:223-6.
- Kc G, Murugesan M, Nayanar SK, Malodan R, Padmanaban M. Comparison of ABO antibody levels in apheresis platelets suspended in platelet additive solution and plasma. *Hematol Transfus Cell Ther.* 2021;43:179-84.
- Tendulkar AA, Jain PA, Velaye S. Antibody titers in Group O platelet donors. *Asian J Transfus Sci.* 2017;11:22-7.
- Bazigou F, Lempesopoulos K, Kavallierou L, Cheropoulou A, Mouratidou M, Baliaga S, et al. Evaluation of anti-A and anti-B alloisogglutinin titer in group O plateletpheresis donors. *Hematol Transfus Int J.* 2015;1:76-81.

20. Josephson CD, Castillejo MI, Grima K, Hillyer CD. ABO-mismatched platelet transfusions: strategies to mitigate patient exposure to naturally occurring hemolytic antibodies. *Transfus Apher Sci.* 2010;42:83-8.
21. Dunbar NM. Does ABO and RhD matching matter for platelet transfusion? *Hematology Am Soc Hematol Educ Program.* 2020;2020:512-7.
22. Berséus O, Boman K, Nessen SC, Westerberg LA. Risks of hemolysis due to anti-A and anti-B caused by the transfusion of blood or blood components containing ABO-incompatible plasma. *Transfusion.* 2013;53(Supple 1):114S-23S.
23. Tynuv M, Flegel WA. Quality improvement with platelet additive solution for safer out-of-group platelet transfusions. *Immunohematology.* 2019;35:108-15.