

Original article

ABO antibody titers and hemolysins in groups O, A, and B blood donors and their pooled leukocyte-poor platelet concentrates at Siriraj Hospital

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Abstract:

Introduction: ABO antibodies may cause severe hemolytic transfusion reactions in ABO-incompatible recipients of whole blood and platelets. **Objective:** To study the ABO antibody titers of groups O, A, and B blood donors and their pooled leukocyte-poor platelet concentrates (LPPC) in platelet additive solution (PAS), the association of ABO antibody titer and hemolytic-inducing ability, and the prevalence of “low antibody titer” group O blood donors at Siriraj Hospital. **Materials and Methods:** One hundred blood donors from each group (O, A, and B) were enrolled. ABO antibody at room temperature (RT) and anti-human globulin (AHG) phases of donors and LPPC were assessed and compared. Hemolytic-inducing ability of donor serum was also evaluated. **Results:** In donors, anti-A and anti-B titers ranged from 4-1,024 and 4-2,048, respectively. In LPPC, anti-A and anti-B titers ranged from 4-128 and 8-256, respectively. The titers in LPPCs were significantly different from their maximum donors’ titer ($p < 0.001$). Hemolysin was positive in 98%, 83%, and 69% of group O, A, and B donors, respectively. The positive hemolysin had higher RT/AHG anti-A and anti-B titers than the negative group ($p < 0.001$). **Conclusion:** ABO antibody titer < 128 was observed in 22% of group O blood donors, but 98% had positive hemolysin. Eighty-five percent of LPPC from these donors had ABO antibodies titer < 128 . Twenty percent of group O LPPC had titer > 128 . Testing for hemolysin in universal group O whole blood donors and assessing ABO antibody titers in LPPC may increase the transfusion safety of ABO incompatible recipients.

Keywords : ● Conventional tube technique (CTT) ● Pooled leukocyte-poor platelet concentrates (LPPC)
● Platelet additive solution (PAS)

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นิพนธ์ต้นฉบับ

ABO แอนติบอดีไทเทรตและฮีโมโกลินในผู้บริจาคโลหิตหมู่ O, A และ B และในผลิตภัณฑ์เกล็ดเลือดชนิดรวมถุงที่มีเม็ดเลือดขาวปนเปื้อนน้อยที่ผลิต ณ โรงพยาบาลศิริราช

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บทคัดย่อ

บทนำ ABO แอนติบอดีอาจทำให้เม็ดเลือดแดงแตกอย่างรุนแรงในผู้รับเลือดและเกล็ดเลือดต่างหมู่ ABO **วัตถุประสงค์** เพื่อศึกษา ระดับ ABO แอนติบอดีของผู้บริจาคโลหิตหมู่ O, A และ B และเกล็ดเลือดชนิดรวมถุงที่มีเม็ดเลือดขาวปนเปื้อนน้อย (LPPC) ความสัมพันธ์ระหว่างระดับ ABO แอนติบอดีและความสามารถในการกระตุ้นให้เม็ดเลือดแดงแตก และความชุกของผู้บริจาคโลหิตหมู่ O ที่มีระดับแอนติบอดีต่ำ ที่โรงพยาบาลศิริราช **วัสดุและวิธีการ** ประเมินระดับ ABO แอนติบอดีที่อุณหภูมิห้อง (RT) และแอนติฮีมาโกปูลินเฟส (AHG) ในผู้บริจาคโลหิตหมู่ O, A และ B หมู่ละ 100 ราย เปรียบเทียบกับใน LPPC และความสามารถในการกระตุ้นให้เม็ดเลือดแดงแตกของซีรัมผู้บริจาค **ผลการศึกษา** พบว่า anti-A และ anti-B ไตเตอร์ในผู้บริจาคอยู่ระหว่าง 4-1,024 และ 4-2,048 ตามลำดับ ส่วนใน LPPC ไตเตอร์อยู่ระหว่าง 4-128 และ 8-256 ตามลำดับ ระดับแอนติบอดีใน LPPC ต่างจากระดับสูงสุดในผู้บริจาคของ LPPC อย่างมีนัยสำคัญ ($p < 0.001$) พบฮีโมโกลินบวก 98%, 83% และ 69% ในผู้บริจาคหมู่ O, A และ B ตามลำดับ กลุ่มบวกมีระดับ RT/AHG anti-A และ anti-B สูงกว่ากลุ่มลบ ($p < 0.001$) **สรุป** ร้อยละ 22 ของผู้บริจาคโลหิตหมู่ O มีระดับแอนติบอดีไทเทรต < 128 แต่ร้อยละ 98 มีฮีโมโกลินเป็นบวก ร้อยละ 85 ของ LPPC จากผู้บริจาคเหล่านี้มีระดับแอนติบอดีไทเทรต < 128, 20% ของ LPPC หมู่ O มีระดับแอนติบอดีไทเทรต > 128 การตรวจฮีโมลิซินในเลือดครบส่วนหมู่ O และ ระดับ ABO แอนติบอดีใน LPPC หมู่ O อาจช่วยเพิ่มความปลอดภัยในการให้เลือดต่างหมู่

คำสำคัญ : ● วิธีการทดสอบแบบหลอดทดลองมาตรฐาน ● เกล็ดเลือดชนิดรวมถุงที่มีเม็ดเลือดขาวปนเปื้อนน้อย

● น้ำยาเลี้ยงเกล็ดเลือด

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2567;34:81-9.

Introduction

ABO antibodies are unique in that they are naturally occurring in each individual according to ABO blood type and are very important to determine safe transfusion. ABO antibodies can cause severe hemolysis or even fatal transfusion reaction if each individual is transfused with ABO-incompatible red cells or plasma¹. This is why every transfusion of red cells and plasma-containing blood products must be selected based on the ABO blood group of the patient and the products. Currently, with the progress of blood component production, it is safe to transfuse ABO-compatible red cells. However, to transfuse ABO non-identical whole blood, the blood bank needs to check the ABO antibody titers in the plasma of whole blood².

At present, the ABO antibody titers are tested in many clinical practices such as ABO incompatible kidney transplantation, emergency transfusion of low-titer group O whole blood (LTOWB), and transfusion of incompatible platelets. All of these applications had different ABO antibodies level that were defined as "low titer". For example, in kidney transplant, low titer was defined as $\leq 512^3$ while for the use of whole blood, the titer in LTOWB varied from 50 to 200⁴. In addition, the low titer for platelet transfusion was defined as $\leq 64^5$. This is why we are interested in testing ABO antibody titers in our blood donors and pooled platelet products.

For platelet transfusion, the platelet products originally contained a considerable amount of plasma, so the ABO antibodies in platelets can cause hemolysis in the recipient. Therefore, the ideal transfusion is ABO-identical platelet. There were reports of fatal cases after transfusion of ABO incompatible plasma which was related to high titer ABO antibodies⁶. However, in large hospitals with high demand for platelet transfusion to support surgery, hematologic patients, trauma, and massive transfusion, the need to transfuse ABO incompatible platelets is not uncommon. Platelet products can be produced by two

major methods. First is the separation of platelets from donated whole blood and pooled the platelets from four to six donors to achieve one adult therapeutic dose. The other is the selective collection of platelets from one donor using an automated blood cell separator. Both types of products contain a large amount of plasma. The difference is the plasma of single donor platelet products come from only one donor, while that of whole blood-derived platelets come from several donors so the risk of having high final antibody titer is lower⁶. This is important because the hemolytic-inducing ability of antibodies is believed to be associated with high antibody titer⁷. Recently, the plasma in pooled platelet product is partly (20-80%) replaced with platelet additive solution therefore the ABO antibody titers should further be reduced⁸. The LPPC is the main platelet product used at Siriraj Hospital and in 2023, all the LPPCs at Siriraj Hospital were produced by replacing two-thirds of the plasma volume with platelet additive solution to reduce the antibody titers and better preserve the platelets. However, whether the final antibody level is lowered enough for a safe transfusion is unknown. It is interesting to explore the final ABO antibody titer in an LPPC unit. Moreover, examining how the final antibody level in LPPC related to the original antibody levels of the 4 donors is noteworthy. In the past, hemolytic-inducing activity or hemolysin was checked prior to the release of group O whole blood for transfusion to the different ABO group recipients. Later, this test is rarely performed in concordance with less whole blood issued from blood bank for transfusion purposes. However, the use of low-titer group O whole blood recently become convincing⁹. Therefore, besides the antibody titers in both donors and LPPC, the hemolytic-inducing ability of antibodies and its association with the antibody titer was also analyzed in this study. The incidence or possible number of whole blood group O with low titers among the donors of Siriraj Hospital was evaluated.

Materials and Methods

This study was approved by the Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University (653/2022). A total of 300 healthy donors were included in this study, including 100 donors with each blood group (A, B, and O) aged from 18-66 years old were consented and enrolled from September to December 2022 at Siriraj Hospital Blood Donation Center. EDTA and clotted blood samples were collected from the diversion pouch of the blood donation set. The whole blood was separated into blood components by the standard protocol of a top-bottom bag system (Teruflex, Terumo BCT, Ltd., Lakewood, Colorado, USA) using an automated presser. The four buffy coats from the same ABO group were pooled together and platelet additive solution (T-PAS, Terumo BCT, Ltd., Lakewood, Colorado, USA) was added before centrifugation to separate the platelet from the white blood cells. These final products were LPPC-PAS or so-called LPPC and the samples from the final products were collected from integrating segments without compromising the closed system of the product. The specified sample sizes were employed for both donors and LPPC for this current pilot study. We included only 20 LPPCs produced from 80 donors in each group because some whole blood donations may not qualify for platelet production, for example, if the collection time exceeds 12 minutes or if there are positive infectious markers. Twenty LPPC of each group (O, A and B) were utilized in this study. ABO antibody titers were determined by the conventional tube test as described in Association for the Advancement of Blood & Biotherapies (AABB) technical manual¹⁰. The standard A₁ cells and B cells from the National Blood Centre, Thai Red Cross Society (The National Blood Centre, Thai Red Cross Society, Bangkok, Thailand) were used in this study. The endpoint of the reaction was 1+. The hemolytic-inducing activity or hemolysin of serum from each donor was tested by incubation of fresh serum with standard A₁ cells or B cells at 37 degrees Celsius and observed for hemolysis¹¹. For statistical analysis,

descriptive statistics were used for demographic data analysis and Mann Whitney non-parametric test were used for the comparison of ABO antibody titers and hemolysins, while the Chi-Square test was used for comparison of ABO antibody titer between the blood donors and LPPC.

Results

There were 198 males and 102 females, with an average age of 39.44 years ranged from 18 to 66 years. The mean ages in groups O, A, and B participants were 40.02±11.59, 38.18±11.24, and 40.13±12.06 years, respectively. The difference in mean age of participants had no statistical significance among donors of the O, A, and B blood groups (groups O and A; $p = 0.947$, group O and B; $p = 0.239$, and groups A and B; $p = 0.258$).

The distribution of ABO antibody titers in blood donors reacting at RT and AHG phases were shown in Figure 1. The anti-A titers of group O at RT and AHG phases were statistically different from those in group B (RT $p = 0.046$ in Figure 1A, AHG $p = 0.000$ in Figure 1B). However, anti-B titer of group O was statistically different from group A only at the AHG phase ($p = 0.000$ in Figure 1D) but not at the RT phase ($p = 0.241$ in Figure 1C). The highest RT and AHG anti-A titers were 1,024 in group O and 2,048 in group B donors, respectively. While the highest RT and AHG anti-B titers were 2,048 in group A and 2,048 in group O, respectively. Comparison of anti-A and anti-B titers at both RT and AHG phases of the donors group O, A, and B were demonstrated in Table 1. In group A and B donors, the median of RT antibody titers was higher than AHG antibody titers (128 vs. 32, RT vs. AHG for group A, 32 vs. 16, RT vs. AHG for group B). Only two group A donors had AHG anti-B higher than RT anti-B titer and six group B donors had AHG anti-A higher than RT anti-A titer. However, the finding of group O donor was completely different. A significant number of group O donors had AHG titer higher than RT (64 vs. 128; RT vs. AHG). Forty five of group O donors had AHG

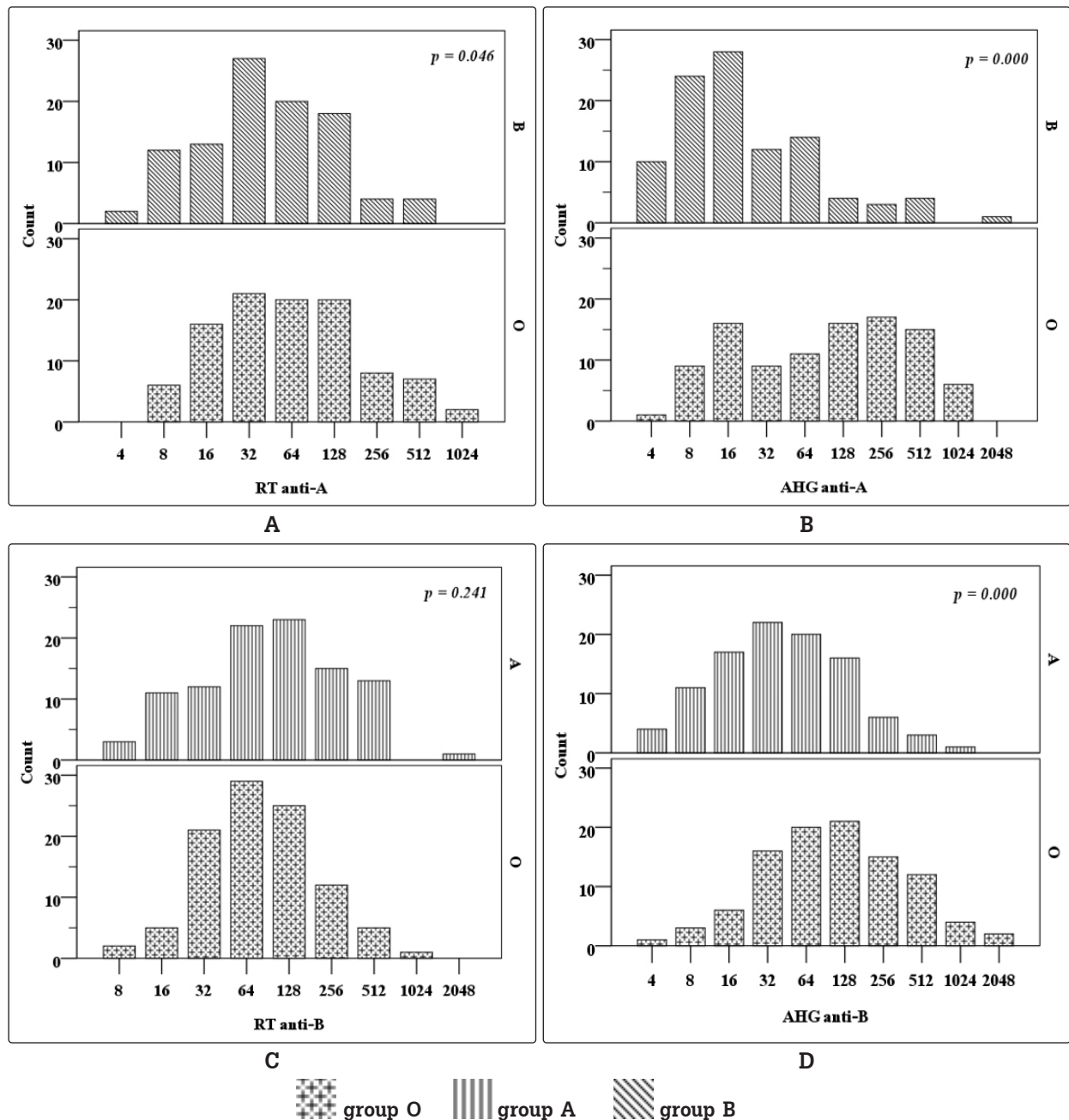


Figure 1 Distribution of ABO antibody titers in groups O, A, and B donors; (1A): RT anti-A in blood group B vs. O, (1B): AHG anti-A in blood group B vs. O, (1C): RT anti-B in blood group A vs. O, (1D): AHG anti-B in blood group A vs. O

Table 1 Comparison of ABO antibody titers between groups O, A, and B (n = 100 for each blood group donor)

Antibody	Min-Max	Median (IQR)	Min-Max	Median (IQR)	p-value
	Group O		Group A		
RT anti-B	8-2,048	64 (32-128)	8-1,024	128 (32-256)	0.241
AHG anti-B	4-1,024	128 (32-256)	4-2,048	32 (16-128)	0.000
	Group O		Group B		
RT anti-A	8-1,024	64 (32-128)	4-512	32 (16-128)	0.046
AHG anti-A	4-1,024	128 (16-256)	4-2,048	16 (8-64)	0.000

RT = room temperature; AHG = anti-human globulin; IQR = interquartile range

anti-B titer higher than RT anti-B titer while 37 group O donors had AHG anti-A titer higher than RT anti-A titer. Groups A and B had a good correlation between RT and AHG antibody titers ($r = 0.863$ for group A, $r = 0.720$ for group B) but there was a weak correlation between ABO antibody titers at RT and AHG phase for group O ($r = 0.316$ anti-B, $r = 0.348$ anti-A).

The frequency of blood donors in group O with anti-A and anti-B titers lower than 256, 128, 64, and 32 was 48%, 22%, 10% and 3%, respectively, as shown in Table 2. The ABO antibody titers at both RT and AHG phase in LPPC ranged from 4 to 256. They were demonstrated and compared in Table 3. The ABO antibody titers more than 64 were observed in 4 group O LPPCs, including 3 donors with a titer of 128 and 1 donor with a titer of 256 and in 1 group A LPPC (RT

anti-B =128). Overall, the group A, B, and O LPPCs with ABO antibody titers more than 64 were observed in 5 LPPCs from a total of 60 LPPCs (8.33%). Table 4 summarized the distribution of ABO antibody titers in the tested LPPC products.

The hemolysin test was positive in 98 from 100 group O donor serum. The two negative hemolysin group O donors were both female (aged 53 and 51 years). The first case had RT and AHG anti-A titers at 8 and 16, respectively, while those of anti-B titers were 16 and 16, respectively. The second case had RT and AHG anti-A titers at 16 and 16, respectively, whereas those of anti-B titers were 64 and 64, respectively. Among group A and B donors, hemolysins were positive in 83% and 69%, respectively. Comparison of ABO antibody titers and hemolysins was shown in Table 5.

Table 2 Frequency of the low titer anti-A and anti-B in blood donor group O (n = 100)

Antibody	Titer < 256 (%)	Titer < 128 (%)	Titer < 64 (%)	Titer < 32 (%)
RT anti-A	83	63	43	22
RT anti-B	82	57	28	7
AHG anti-A	64	46	35	26
AHG anti-B	67	46	26	10
RT and AHG anti-A	60	43	29	16
RT and AHG anti-B	62	37	14	4
RT and AHG anti-A and anti-B	48	22	10	3

RT = room temperature; AHG = anti-human globulin; IQR = interquartile range

Table 3 ABO antibody titers in LPPCs

Antibody	Blood group	Antibody titer		
		Min-Max	Median (IQR)	p-value
RT anti-A	O	8-64	32 (16-64)	0.006
	B	4-64	16 (12-24)	
AHG anti-A	O	16-128	32 (32-64)	0.000
	B	4-32	12 (6-16)	
RT anti-B	O	8-128	32 (16-48)	0.964
	A	8-128	32 (16-48)	
AHG anti-B	O	16-256	32 (16-64)	0.019
	A	8-64	16 (8-24)	

RT = room temperature; AHG = anti-human globulin; IQR = interquartile range

Table 4 Frequency of LPPCs group O, A, and B at different ABO antibody phases and titers

Titer	Group O (n = 20)		Group O (n = 20)		Group B (n = 20)		Group A (n = 20)	
	Anti-A RT	Anti-A AHG	Anti-B RT	Anti-B AHG	Anti-A RT	Anti-A AHG	Anti-B RT	Anti-B AHG
4	0	0	0	0	1	5	0	0
8	3	0	1	0	4	5	2	9
16	4	4	6	7	10	8	4	6
32	6	8	8	4	4	2	9	2
64	7	5	4	7	1	0	4	3
128	0	3	1	1	0	0	1	0
256	0	0	0	1	0	0	0	0

RT = room temperature; AHG = anti-human globulin

Table 5 Comparison of ABO antibody titers and hemolysins in donor groups A and B

Group	Antibody reaction	Hemolysin positive	Hemolysin negative	p-value
	phase	median (IQR)	median (IQR)	
A	RT anti-B	128 (64-256)	32 (16-64)	0.007
	AHG anti-B	64 (32-128)	8 (8-32)	0.022
B	RT anti-A	64 (32-128)	16 (8-32)	0.000
	AHG anti-A	16 (16-64)	8 (4-16)	0.082

RT = room temperature; AHG = anti-human globulin; IQR = interquartile range

Discussion

We studied RT and AHG anti-A and anti-B titers, and hemolysins in individual donor and ABO antibodies in pooled leukocyte-poor platelet concentrates that were produced from these donors. The ABO antibody titers in blood donors varied from 4-2,048. The hemolysins were positive in 98% of group O donors, 83% of group A and 69% of group B donors. ABO antibody titers in LPPCs were significantly lower than that in blood donors.

Mavichak W et al¹² studied the room temperature reacting antibodies in 100 group O blood donors and they found that the anti-B was higher than the anti-A which was consistent with our finding (Table 1). The maximum titer of RT anti-A in our study was only one-fold higher than Mavichak et al study (1,024 vs. 512)¹². However, we found that most of our median titers of donors group O, A and B revealed one titer lower than the results from Onpuns S, et al. except for RT anti-B in group A. The median titers of anti-A and anti-B in group O in our study and Onpuns study was higher

than the titer in group A or B¹³. Mavichak et al reported only RT titer anti-A and anti-B ≤ 64 in 84% and 73% of group O donors, respectively¹², which is a higher number than our study (43% of RT titer anti-A and 28% RT titer anti-B). The study conducted by Park ES, et al. in Korea revealed lower titers for both RT and AHG anti-A and anti-B compared to ours. In Park ES, et al.'s study, the median titer ranged from 8 to 32, whereas in our study, the median titer ranged from 16 to 128. The difference in antibody titer among these works may be a result of the different ethnicity and population that was included in the studies¹⁴. In the present study, group O exhibited significantly higher reactivity of ABO antibodies at both RT and AHG compared to group B. The highest ABO antibody titer (2,048) was observed in group O (AHG anti-B), group A (RT anti-B) and group B (AHG anti-A). The incidence of low-titer group O (< 128) was observed in 22% and if we looked for an ABO titer less than 64, this was presented in 10%. The latter frequency was fairly similar to the findings in

the United States by Zielinski M, et al. and Seheult J, et al. that reported the frequencies of group O low-titer donors (< 100) as 8% and 10%, respectively^{15,16}.

There were limited studies about hemolysins in blood donors. Khampanon, et al.¹⁷ studied 300 group O blood donors and found that only 69% of them had positive hemolysins. Moreover, 34% of them were hemolysin positive of both anti-A and anti-B. Interestingly, in our study, 98% of our group O donor had hemolysin positive which was much more than Khampanon finding. For group A and B donors, the hemolysin was positive in 83% and 69%, respectively. When correlating the result of hemolysins with the ABO antibody titers, it was discovered that the negative hemolysins group had lower ABO titers than the positive group. This was agreed with the finding from Bastos EP, et al¹¹. Therefore, the idea of association between hemolytic-inducing ability of antibodies and the high antibody titers is confirmed. Nevertheless, we detected positive hemolysin in donors from groups A and B, even with RT titers as low as 8 and AHG titers as low as 4. Consequently, it is inferred that there are additional factors influencing hemolysis beyond ABO titers. Since the positive hemolysin test may cause hemolysis, it should be included in the high-titer group regardless of the antibody level. So, we suggest that for safe ABO minor incompatible blood transfusion, hemolysin test should be performed. In addition, the fact that hemolysin positive might not be relevant the intensity of antibody titer supports the idea that the hemolysin test may be useful to identify the suitable group O whole blood.

There is no consensus about the ABO antibody titer that was safe for transfusion of ABO incompatible platelets. However, there were some reports about hemolysis after transfusion of ABO incompatible pooled platelets. One of those had investigated the antibody titer and revealed the very high anti-A at 37°C in both donor (4,096) and platelet product (2,048)¹⁸. The other reports of acute hemolysis in recipients who received single donor platelets with anti-A and anti-B titer of

128⁶. So, the blood center must be very careful when allowing the transfusion of ABO incompatible platelets. For this choice of platelet transfusion, the situation must be reasonably urgent and the products should be tested to safeguard against over-hemolysis. In our LPPC production process, such a small number of pooled platelets that had ABO antibodies titer exceeding 128 were obtained. Group O LPPC has higher anti-A, and anti-B titers than groups A and B. There was no available data to compare ABO antibody titer in LPPC because even regular LPPC (without PAS) was considered safe for transfusion to ABO incompatible recipient. However, we still recommended that the group O LPPC may be tested for target ABO antibodies titer to ensure the safety of platelet transfusion.

Conclusion

ABO antibodies in blood donors varied from 4 to 2,048 in our study. There was no consensus definition for low-titer of whole blood and platelets. However, to provide safe blood for transfusion, the blood center needs to be very careful about how to determine the low ABO titer and decides about the safest practice which is feasible for our donors and our platelet products. In addition, hemolysin test may be useful for identifying the suitable group O whole blood as a universal group O low-titer for every ABO recipient. In our study, the qualified group O low titer may be as low as 2%. For platelet transfusion, adding PAS to LPPC results in a lower ABO antibodies titer, however, 20% of LPPC group O in PAS still had ABO antibodies exceeding 128. We recommend testing at least for the target ABO titer for group O platelets which will be used for ABO-incompatible recipients to avoid hemolysis. However, both of our suggestions for the purpose of possible hemolysis risk reduction in testing hemolysin for suitable LTOWB and antibody titer for group O LPPC derived from only *in vitro* study. The consequence in recipients has not been monitored. Therefore, to prove whether our recommendations are worth to follow, the future *in vivo* study should be investigated.

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