ANESTHESIOLOGY

# Transfusion of Uncrossmatched Group O Erythrocyte-containing Products Does Not Interfere with Most ABO Typings

Mark H. Yazer, M.D., Philip C. Spinella, M.D., Leilani Doyle, M.D., Richard M. Kaufman, M.D., Robyn Dunn, B.S., John R. Hess, M.D., Luiz Amorim Filho, M.D., Magali Fontaine, M.D., Birgit Gathof, M.D., Bryon Jackson, M.D., Michael F. Murphy, M.D., Jeremiah Pasion, M.D., Jay S. Raval, M.D., Kristin Rosinski, M.L.T., Jansen Seheult, M.D., Andrew W. Shih, M.D., Jason Sperry, M.D., Julie Staves, B.Sc. (Hons), Erin E. Tuott, B.S., Alyssa Ziman, M.D., Darrell J. Triulzi, M.D., on behalf of the Biomedical Excellence for Safer Transfusion Collaborative*

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## EDITOR'S PERSPECTIVE

### What We Already Know about This Topic

- Uncrossmatched erythrocyte units that are provided to a massively bleeding patient whose ABO group is unknown must be type O to ensure compatibility. The effect of transfusing type O units on the ability to subsequently determine the patient's ABO group is not known.

### What This Article Tells Us That Is New

- ABO typing in 665 of 695 (95.7%) non–group O recipients could be accurately determined on the first type and screen sample obtained by the blood bank after the transfusion of uncrossmatched type O erythrocyte-containing products.

## ABSTRACT

**Background:** Group O erythrocytes and/or whole blood are used for urgent transfusions in patients of unknown blood type. This study investigated the impact of transfusing increasing numbers of uncrossmatched type O products on the recipient's first in-hospital ABO type.

**Methods:** This was a retrospective cohort study. Results of the first ABO type obtained in adult, non–type O recipients (*i.e.*, types A, B, AB) after receiving at least one unit of uncrossmatched type O erythrocyte-containing product(s) for any bleeding etiology were analyzed along with the number of uncrossmatched type O erythrocyte-containing products administered in the prehospital and/or in hospital setting before the first type and screen sample was drawn.

**Results:** There were 10 institutions that contributed a total of 695 patient records. Among patients who received up to 10 uncrossmatched type O erythrocyte-containing products, the median A antigen agglutination strength in A and AB individuals on forward typing (*i.e.*, testing the recipient's erythrocytes for A and/or B antigens) was the maximum (4+), whereas the median B antigen agglutination strength among B and AB recipients of up to 10 units was 3 to 4+. The median agglutination strength on the reverse type (*i.e.*, testing the recipient's plasma for corresponding anti-A and -B antibodies) was very strong, between 3 and 4+, for recipients of up to 10 units of uncrossmatched erythrocyte-containing products. Overall, the ABO type of 665 of 695 (95.7%; 95% CI, 93.9 to 97.0%) of these patients could be accurately determined on the first type and screen sample obtained after transfusion of uncrossmatched type O erythrocyte-containing products.

**Conclusions:** The transfusion of smaller quantities of uncrossmatched type O erythrocyte-containing products, in particular up to 10 units, does not usually interfere with determining the recipient's ABO type. The early collection of a type and screen sample is important.

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To provide the safest possible transfusion, it is imperative that the patient's ABO type is known and that their plasma is tested for the presence of unexpected erythrocyte antibodies that might have been formed after previous transfusions or pregnancies. Collectively, determining the recipient's ABO type and testing their plasma for antibodies to erythrocyte antigens is referred to as a type (or group) and

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*Members of the Biomedical Excellence for Safer Transfusion Collaborative are listed in the appendix.

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screen.[1] However, as a type and screen can take up to an hour to complete after the sample is received by the blood bank,[1] it is clinically detrimental to wait until the testing is complete to provide blood products to a bleeding patient. Because the ABO type of these patients is not likely to be available at the time that transfusions are initiated either prehospital or early in their in-hospital course, type O erythrocytes are the only erythrocytes that can be safely transfused in this situation. When administered before the patient's type and screen test has been completed, these type O erythrocytes are known as uncrossmatched erythrocytes. A recent literature review found a 0.1% rate of hemolysis among bleeding patients who received uncrossmatched erythrocytes[2]; thus, they are safe to administer to patients whose survival would be jeopardized by waiting until the results of the type and screen are known.

Although uncrossmatched type O erythrocytes should never be denied to a massively bleeding patient whose ABO type is unknown, one consideration after their administration is whether the transfusion of the type O erythrocytes will interfere with the blood bank's ability to subsequently determine the recipient's ABO type. That is, if a recipient is type A, B, or AB, will the transfusion of type O erythrocytes "mask" their true ABO type and require the continued use of type O erythrocytes? Type O erythrocytes are a very limited resource that are often in high demand because of their universal compatibility,[3] so preserving the blood bank's inventory for the group O patients who can only receive group O erythrocyte units is very important. Being able to provide type-specific erythrocytes to patients who are not group O is an important way of preserving the inventory of group O erythrocytes. The objective of this study was to determine the effect of transfusing various quantities of uncrossmatched type O erythrocytes on the ABO and D types obtained on the first type and screen sample submitted to the blood bank after beginning the resuscitation.

## Materials and Methods

Members of the Biomedical Excellence for Safer Transfusion (BEST) collaborative and colleagues from trauma centers who were not members of BEST were invited to participate in this retrospective, multicenter, cohort study. Participants were asked to retrospectively identify non–type O (*i.e.*, types A, B, and AB) patients who received at least one unit of uncrossmatched type O erythrocyte-containing products for any reason such as trauma resuscitation, bleeding in the operating room, gastrointestinal bleeding emergencies, *etc.*, in calendar years 2015 through 2018. Type O erythrocyte-containing products included conventional erythrocyte units and low-titer group O whole blood units in any combination. The inclusion criteria for this study included: (1) age more than 18 yr old and (2) had at least one type and screen sample collected and tested, with the strength of the agglutination on forward and reverse typing available for analysis. The exclusion criterion for this study was the receipt of type O erythrocyte and/or low-titer group O whole blood transfusions in the 120 days preceding the index transfusion of uncrossmatched type O erythrocyte-containing products because it would not have been possible to differentiate any effect on the type and screen sample caused by the previously transfused group O erythrocytes. D+ and D− recipients of uncrossmatched type O erythrocyte-containing products were eligible for inclusion, and the uncrossmatched group O erythrocyte-containing products themselves could have been D+ or D−.

For eligible patients, the number and D type (*i.e.*, D+ or D−) of uncrossmatched type O erythrocyte-containing products and the number and ABO type of plasma and platelet units that were transfused before the sample for the first type and screen were collected. Erythrocyte-containing products that were transfused after the collection of the sample for the first type and screen sample were not included. The nature and quantity of any blood products that were administered in the prehospital phase of the resuscitation were included. Platelet transfusions were recorded as the number of doses administered (either single donor units or pools of random donor platelets), and "jumbo" or double apheresis plasma units were counted as two plasma units.

To determine a patient's ABO type, two complementary tests are routinely performed.[2] A "forward" type involves separately mixing the recipient's erythrocytes with commercially available anti-A, anti-B, and anti-D testing reagents and then determining whether agglutination, *i.e.*, erythrocytes clumping together, occurs (fig. 1A). The strength of the agglutination is graded as 0 (no agglutination, a negative result) through 4+ (maximum agglutination; a single clump of erythrocytes is observed). The term "weak" is used to describe a positive agglutination strength that is less than 1+. The "reverse" type involves mixing the recipient's plasma with commercially available $A_1$ and B erythrocytes and then determining whether agglutination occurs (fig. 1B). The same 0 to 4+ scale, including the "weak" strength, is used to grade the agglutination on the reverse type. Most laboratories require the agglutination strength on the forward and reverse types to be at least 2+ to establish the blood type; if weaker than 2+ agglutination is observed on either the forward or reverse types, the blood group is usually left as indeterminate until future samples demonstrate sufficiently strong agglutination strength. The forward and reverse typing tests should be complementary; for example, a group A recipient's erythrocytes should demonstrate at least 2+ agglutination with the anti-A testing reagent on the forward type, and their plasma should demonstrate at least 2+ agglutination with the B cells on the reverse type.

Mixed field agglutination can occur when a type A, B, or AB (*i.e.*, non–type O) recipient is transfused with type O erythrocytes; on the forward type, some cells would appear agglutinated, whereas others would not be agglutinated (fig. 1C). For example, in a group A recipient of group O erythrocytes, when the anti-A reagent is added to their erythrocytes in the forward type, the patient's group A erythrocytes would agglutinate, whereas the donor group O erythrocytes would not agglutinate, thereby giving the

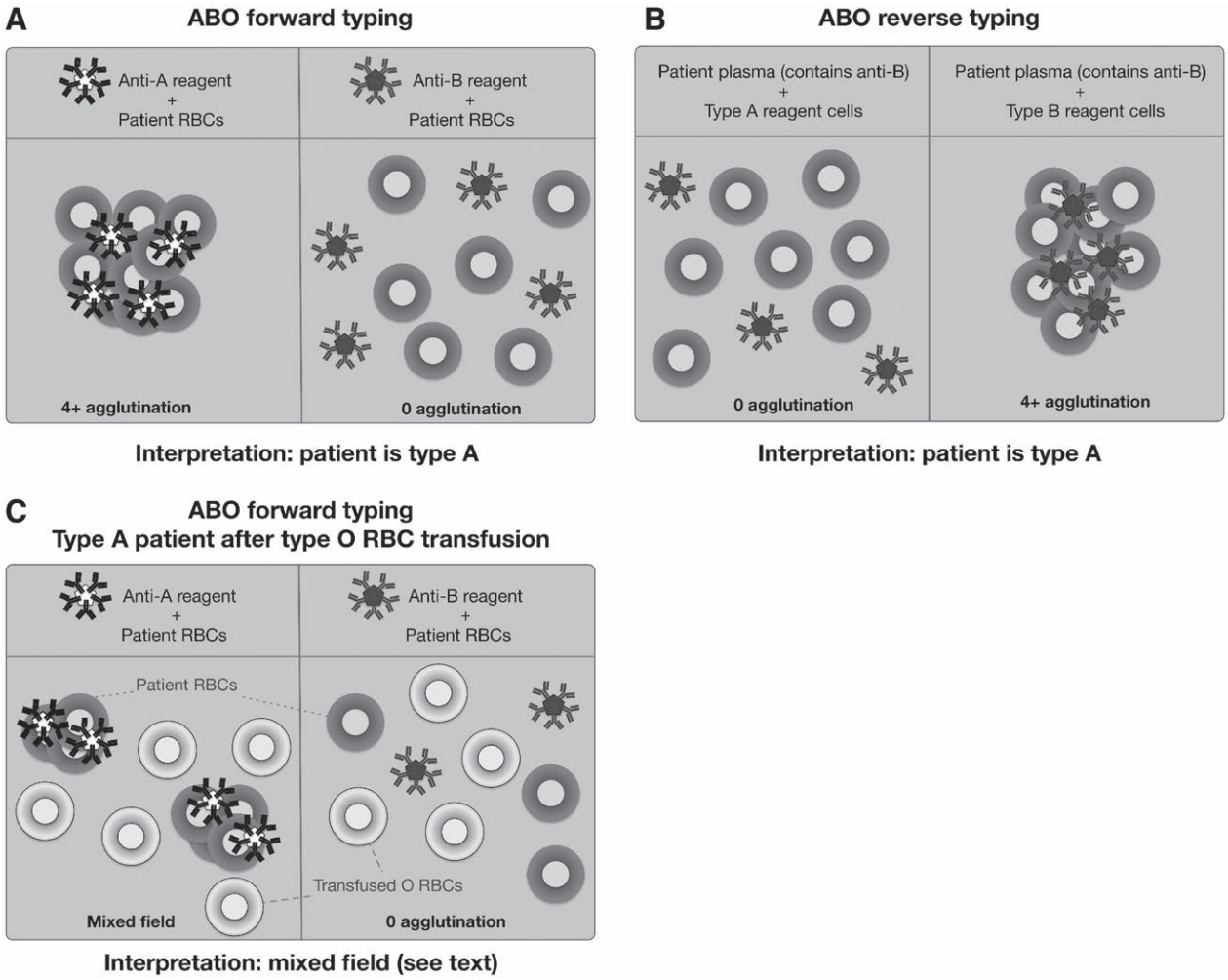


**Fig. 1.** Depiction of a forward type (*A*), reverse type (*B*), and mixed field agglutination in a type A patient (*C*). When the anti-A reagent is added, it causes the patient's group A erythrocytes to agglutinate, whereas adding anti-B reagent does not cause the patient's group A erythrocytes to agglutinate. When group O erythrocytes are transfused to the group A recipient and anti-A reagent is added, the patient's group A erythrocytes agglutinate, but the transfused group O erythrocytes do not agglutinate, thereby creating a mixed field appearance. RBC, erythrocyte (red blood cell).

appearance of two distinct erythrocyte populations in the sample. Thus, the appearance of mixed field agglutination can complicate the determination of the ABO type because two different types of erythrocytes are detected in the same recipient. Some blood banks have a policy whereby if it is known that an A, B, or AB patient has received type O erythrocytes, the mixed field can be ignored if the strength of the agglutinated erythrocytes would otherwise meet the criteria for establishing the ABO type; at these centers, the explicable presence of mixed field agglutination would not invalidate the ABO type. Mixed field agglutination is never detected on the reverse typing as the patient's plasma, not erythrocytes, is used to perform this test.

The agglutination strengths of the forward and reverse typings, as well as the presence of mixed field agglutination, were documented for each patient in this study. If a patient did not have the agglutination strength on the forward typing recorded because of the presence of mixed field agglutination, they were excluded from this study. For the reverse typing, if techniques such as 4°C incubation or enzyme treatment were performed to enhance weak agglutination, the preenhancement agglutination strength was reported. The method by which each patient's sample was tested was also recorded.

## *In Vitro* Mixing Study to Predict the Occurrence of Mixed Field Agglutination

An *in vitro* mixing study was performed to simulate the effect of transfusing different volumes of type O, D+ allogeneic erythrocytes to a non–type O, D− recipient. Erythrocytes from a donated group AB− allogeneic erythrocyte unit

were mixed with increasing quantities of erythrocytes from a donated group O+ allogeneic erythrocyte unit, and the A and B agglutination strengths, as well as the frequency of occurrence of mixed field agglutination, were determined using the manual tube method. Agglutination was scored using the 0 to 4+ scale, and mixed field was noted when it was detected.

### Statistical Analysis

No statistical power calculation was conducted before the study, and the sample size was based on the available data. The data are presented as median (95% CI, or interquartile range), and the differences between categorical variables were evaluated using the chi-square test (Excel 2010, Microsoft, USA; and Prism version 7, GraphPad Software, USA). The chi-square test for trends in proportions was used to test for a linear trend in the percent of recipients with less than 2+ agglutination strengths (invalid ABO types) across the number of uncrossmatched type O erythrocyte-containing products transfused (R, version 3.4.2, R Core Team 2017, Austria). Quantile regression was used to compare the median volume of incompatible and type AB plasma and platelets transfused between the group with 1+ agglutination strength on reverse typing and the groups with stronger agglutination strengths, *i.e.*, 2+, 3+, and 4+ (Stata, version 15, Statacorp, USA). An ordered logistic regression and Brant test of the proportional odds assumption were used to model the relationships between the volume of incompatible and type AB plasma and platelets transfused and the agglutination strength on the reverse typing for type A and type B recipients, separately (Stata, version 15, Statacorp, USA).

All the data that were collected for this effort were gathered under research protocols that were fully approved by a local ethics committee or viewed as exempt by a local ethics committee. At each participating site, the local ethics committee granted a waiver of informed consent because of the minimal risk of this project.

**Table 1.** Number of Patient Records Contributed by Each Site

| Site No. | No. of Patients Contributed (% of total) |
|---|---|
| 1 | 187 (26.9) |
| 2 | 77 (11.1) |
| 3 | 50 (7.2) |
| 4 | 26 (3.7) |
| 5 | 38 (5.5) |
| 6 | 99 (14.2) |
| 7 | 10 (1.4) |
| 8 | 22 (3.2) |
| 9 | 176 (25.3) |
| 10 | 10 (1.4) |

Note that institution 5 documented 43 patient records, but 5 were excluded because the presence of mixed field agglutination prevented the recording of the actual agglutination strengths on the patients' forward typing.

## Results

In total, 10 institutions participated in this study. Six were from the United States, and there was one participant from each of Brazil, Canada, England, and Germany. Table 1 demonstrates the number of patients contributed by each site. One institution accrued five patients who were excluded from the analysis because the presence of mixed field agglutination prevented the recording of the exact agglutination strength on forward typing at that institution. There were 695 non–type O patients who received at least one unit of uncrossmatched type O erythrocyte-containing products who met the inclusion criteria: 486 of 695 (69.9%) were group A, 168 of 695 (24.2%) were group B, and 41 of 695 (5.9%) were group AB; 627 of 695 (90.2%) of these recipients were D+, whereas 68 of 695 (9.8%) were D−.

Information on the time between the transfusion of the first uncrossmatched type O erythrocyte-containing product and the drawing of the first sample for a type and screen was available on 486 of 695 (70%) patients; the median time was 23 min (interquartile range, 12 to 45 min). During this period of time, the patients in this study received a median of 2 (interquartile range, 1 to 4; range, 1 to 18) type O erythrocyte-containing products. Overall, the majority of patients in this study (439 of 695, 63.2%) received either one or two unit(s) of uncrossmatched type O erythrocyte-containing products before the sample for the first type and screen sample was collected (fig. 2). The median (interquartile range) number of type O erythrocyte-containing products transfused to the patients who received 5 to 10 units was 6 (5.0 to 8.0) units, whereas that for those who received more than 10 units was 12 (11.3 to 14.0) units. The tube method was used for ABO typing in 298 of 695 (42.9%), the gel method was used in 272 of 695 (39.1%), and the solid-phase method was used in 125 of 695 (18.0%) of patients.

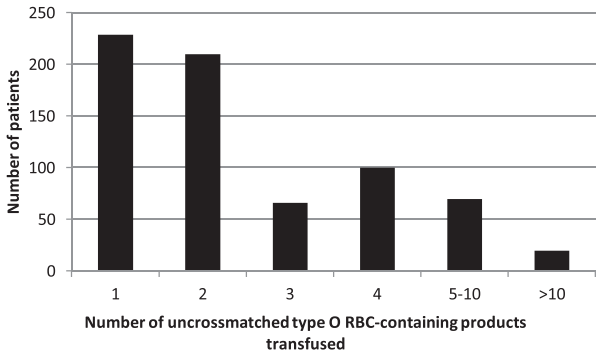


**Fig. 2.** The distribution of uncrossmatched type O erythrocyte-containing products transfused to the 695 patients in this study before the first type and screen sample was collected. RBC, erythrocyte (red blood cell).

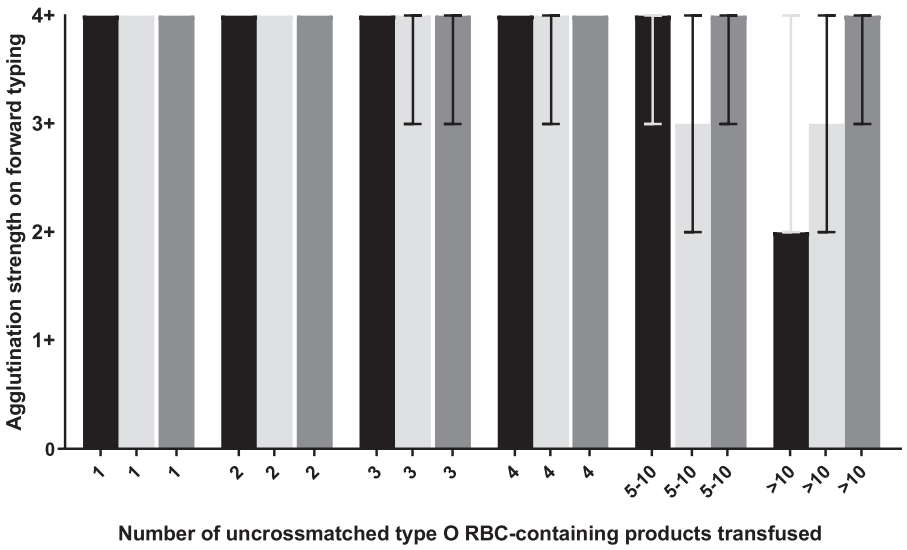


**Fig. 3.** The median (95% CI) agglutination strength on the forward typing stratified by the number of uncrossmatched type O erythrocyte-containing products transfused. RBC, erythrocyte (red blood cell).

## Forward Typing Strengths Based on Number of Type O Erythrocyte-containing Products Transfused

The median (95% CI) agglutination strengths on the forward typing stratified by the number of uncrossmatched type O erythrocyte-containing products is shown in figure 3. Note that because type AB individuals have A and B antigens on their erythrocytes, their A and B agglutination strengths on forward typing are included with the A and B recipients, respectively. The median agglutination strength was the maximum 4+ for anti-A, anti-B, and anti-D when up to 10 uncrossmatched type O erythrocyte-containing products had been transfused, with the exception of the median B antigen agglutination strength of 3+ in the recipients of between 5 and 10 units. Sixty-eight of the patients who received up to 10 uncrossmatched type O erythrocyte-containing products typed as D− and mixed field was detected in the D typing of 124 of these patients.

When patients had received more than 10 units of uncrossmatched type O erythrocyte-containing products, the median A antigen agglutination strength decreased to 2+, whereas the median B antigen agglutination strength was 3+. The median agglutination strength for anti-D was the maximum 4+, and all of these patients typed as D+, although mixed field was detected in the D typing of five of these patients.

## Detection of Mixed Field Agglutination on Forward Typing

The detection of mixed field agglutination in the A and B typings, that is, the presence of two distinct populations of erythrocytes in a recipient's sample such as type A and O erythrocytes in a type A patient who received type O erythrocytes, tended to increase as the number of uncrossmatched type O erythrocyte-containing products increased (fig. 4). There were 129 recipients in this study with mixed field agglutination in their D typing. Of these recipients, 42 of 129 (32.6%) were transfused only with D+ uncrossmatched type O erythrocyte-containing products; thus, the native Rh type of these recipients was D−. There were 72 of 129 (55.8%) recipients with mixed field agglutination detected in their D typing who received only D− uncrossmatched type O erythrocyte-containing products; thus, the native Rh type of these recipients was D+. Of particular interest, there were 15 of 129 (11.6%) recipients with mixed field D typing who received both D+ and D− uncrossmatched type O erythrocyte-containing products. Based on subsequent testing, the actual D typing of 13 of these patients was determined to be D+, whereas the remaining 2 were D−.

## Reverse Typing Strengths Based on Number of Type O Erythrocyte-containing Products Transfused

The median (95% CI) agglutination strength on the reverse typing stratified by the number of uncrossmatched type O erythrocyte-containing products is shown in figure 5. Like the agglutination on the forward typings, the median agglutination strengths on the reverse typings were also strong and ranged between 3 and 4+ for recipients of up to 10 units of uncrossmatched type O erythrocyte-containing products; there was increased variability in agglutination strength especially for the anti-A (*i.e.*, type B) when patients had received 5 to 10 uncrossmatched type O erythrocyte-containing products. Once patients had received more than 10 units, the median agglutination strengths were between 2 and 3+.

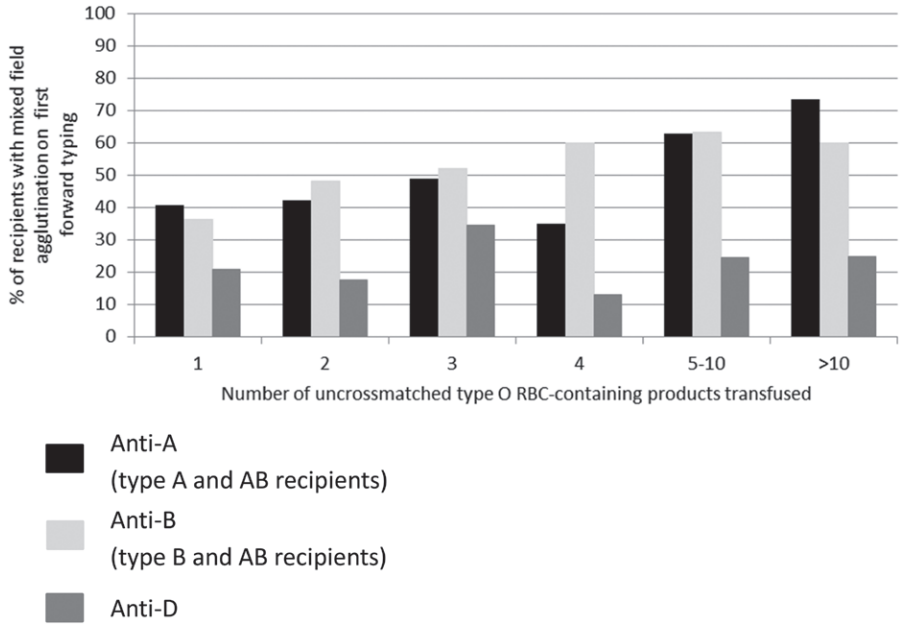


**Fig. 4.** The frequency of detecting mixed field agglutination based on the number of uncrossmatched type O erythrocyte-containing products transfused. RBC, erythrocyte (red blood cell).

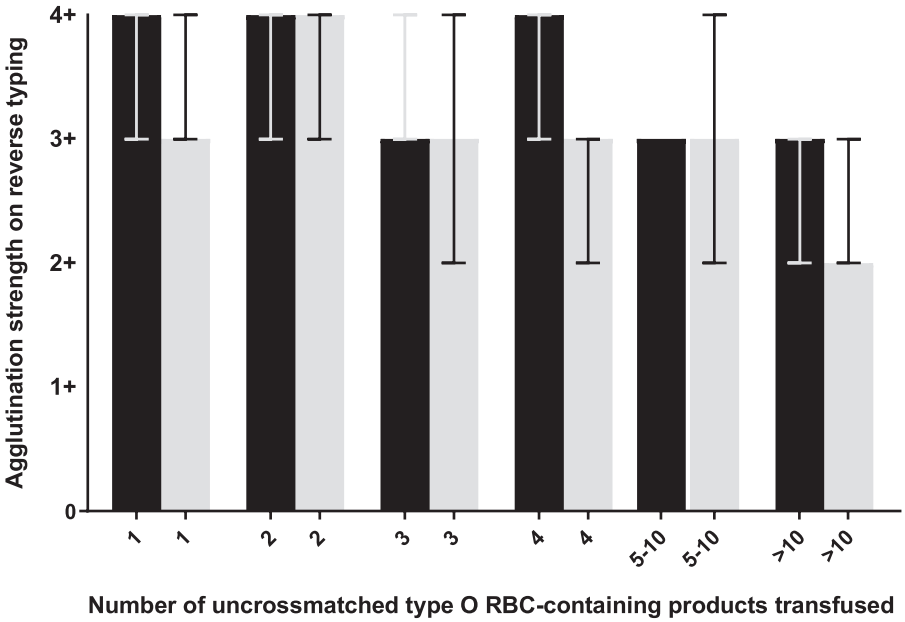


**Fig. 5.** The median (95% CI) agglutination strength on the reverse typing stratified by the number of uncrossmatched type O erythrocyte-containing products transfused. RBC, erythrocyte (red blood cell).

## Dilutional Effect of Transfusing Plasma and Platelets on the Reverse Type

To understand the dilutional effect that transfusing both incompatible plasma and platelets and type AB plasma and platelets might have on the recipient's anti-A or anti-B agglutination strength on the reverse typing, the relationship between the volume of incompatible plasma (including the volume of type AB plasma transfused because

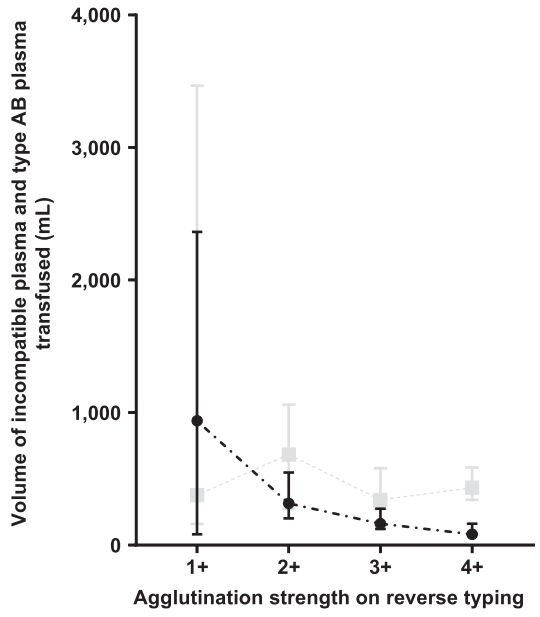


**Fig. 6.** The median (interquartile range) volume of incompatible and type AB plasma and platelets transfused to type A and B recipients stratified by the agglutination strength on the reverse typing.

it does not contain anti-A or anti-B antibodies and also including the volume of plasma contained in any platelet units transfused to each patient) transfused to type A and B recipients and the strength of agglutination on the reverse typing was determined (fig. 6). Note that type AB individuals do not have either anti-A or anti-B in their reverse type and were thus excluded from this analysis (n = 41). There was a relatively small number of type A recipients with the weakest (1+) anti-B agglutination on their reverse type (n = 16); these patients received a larger median volume of incompatible and type AB plasma and platelets compared with those with stronger (2+ n = 86, 3+ n = 138, 4+ n = 246) agglutination ($P < 0.001$ for all pairwise comparisons). For every 100-mL increase in the volume of incompatible and type AB plasma and platelets transfused, the odds of the lowest agglutination strength *versus* the higher agglutination strengths were 1.06 greater (95% CI of odds ratio, 1.03 to 1.08; $P < 0.001$) with no violation of the proportional odds assumption (Brant test chi-square = 1.99, degrees of freedom = 2, $P = 0.370$). However, there appeared to be no relationship between the volume of incompatible and type AB plasma and platelets transfused and the agglutination strength on the reverse typing for the type B recipients (odds ratio = 1.03; 95% CI, 0.99 to 1.07, $P = 0.140$; Brant test chi-square = 0.87, degrees of freedom = 2, $P = 0.647$), although there was considerable variability around the median quantity of plasma transfused and platelets to the recipients with 1+ agglutination (1+ n = 6, 2+ n = 45, 3+ n = 50, 4+ n = 67).

### Insufficiently Strong Agglutination Strengths Lead to the Inability to Determine ABO Type on First Type and Screen Sample

Using the criterion that the agglutination strength on the forward and reverse typings must be at least 2+ to assign an ABO type, there were 30 of 695 (4.3%; 95% CI, 3.0 to 6.1%) patients who had less than 2+ agglutination on their forward and/or reverse typings on the first type and screen sample after receipt of uncrossmatched erythrocytes and whose ABO type could therefore not be determined. Thus, for 665 of 695 (95.7%; 95% CI, 93.9 to 97.0%) of the patients in this study, receipt of uncrossmatched type O erythrocyte-containing products did not complicate the determination of their ABO types. Figure 7 demonstrates the percentage of patients whose ABO type could not be determined from the first type and screen sample because of insufficient agglutination strength on either the forward and/or reverse typing based on the number of uncrossmatched type O erythrocyte-containing products that they received before the sample for the first type and screen was drawn. Although small overall, the percentage of patients who demonstrated less than 2+ agglutination strengths on their forward and/or reverse typings generally increased as the number of transfused uncrossmatched products increased such that 3 of 20 (15%) of the patients who received more than 10 products did not demonstrate sufficient agglutination strength for an ABO type determination (test for trend in proportions: chi-square = 20.91, degrees of freedom = 1, $P < 0.001$).

An invalid ABO group, that is, insufficiently strong agglutination, was detected using the gel technique in 20 of 30 (66.7%) patients, the tube method in 6 of 30 (20.0%) patients, and the solid-phase method in 4 of 30 (13.3%) patients. These frequencies were significantly different than the frequencies at which these tests were used to type the patients ($P = 0.009$).

### *In Vitro* Mixing Study

As increasing quantities of O+ erythrocytes were added to the AB− erythrocytes, the A and B agglutination strengths decreased, while the D agglutination strength increased, and mixed field agglutination was detected more frequently (table 2).

## Discussion

These data indicate that the transfusion of uncrossmatched type O erythrocyte-containing products did not interfere with the ABO type determination for the majority of recipients in this study (665 of 695 [95.7%]; 95% CI, 93.9 to 97.0%). However, the majority of the recipients in this study received fewer than 10 units, and the rate of invalid ABO types increased with the number of transfused products, in particular when patients had received more than 10 products (fig. 7). Thus, obtaining a sample for the type and screen early in the resuscitation is important for accurate

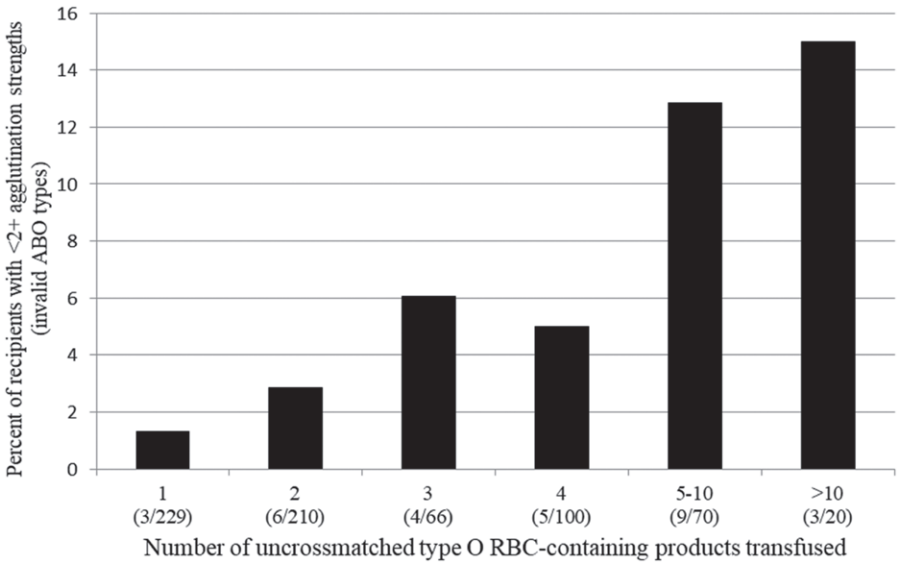


**Fig. 7.** The percentage of recipients whose ABO type could not be determined on the first type and screen sample after receipt of uncrossmatched type O erythrocyte-containing products because of less than 2+ agglutination on forward and/or reverse typings. The *numerator* in *parentheses* represents the number of patients with an invalid ABO type, and the *denominator* represents the total number of patients who received that quantity of uncrossmatched type O erythrocyte-containing products. RBC, erythrocyte (red blood cell).

**Table 2.** *In Vitro* Mixing Study of AB– Erythrocytes with O+ Erythrocytes

| Dilution AB–:O+ | Anti-A | Anti-B | Anti-D |
|---|---|---|---|
| 1:0 | 4+ | 4+ | 0 |
| 1:0.25 | 4+ | 4+ | 0 |
| 1:0.50 | 4+ m/f | 4+ m/f | 0 |
| 1:0.75 | 4+ m/f | 4+ m/f | Weak m/f |
| 1:1 | 4+ m/f | 3+ m/f | 1+ m/f |
| 1:1.5 | 3+ m/f | 3+ m/f | 1+ m/f |
| 1:2 | 3+ m/f | 2+ m/f | 1+ m/f |
| 1:2.5 | 2+ m/f | 2+ m/f | 2+ m/f |
| 1:5 | 1+ m/f | 1+ m/f | 3+ m/f |
| 1:7.5 | Weak m/f | Weak m/f | 3+ m/f |

For example, a 1:1 dilution means that one part AB– erythrocytes was mixed with one part O– erythrocytes. The numbers next to the + sign indicate the agglutination strength, and "weak" indicates that agglutination was detected but was weaker than 1+. m/f, mixed field agglutination was detected.

ABO determination. There were 30 of 695 (4.3%; 95% CI, 3.0 to 6.1%) recipients in this study who would have to have been maintained on type O erythrocytes until a subsequent type and screen sample demonstrated stronger agglutination strengths.

The detection of mixed field agglutination was relatively uncommon until relatively larger quantities of uncrossmatched type O erythrocyte-containing products had been administered. The presence of mixed field agglutination would not interfere with the assignment of the recipient's ABO type in some transfusion services; if the recipient's forward and reverse types demonstrated at least 2+ agglutination strengths and the transfusion of uncrossmatched type O erythrocyte-containing products could be confirmed, the mixed field agglutination would be ignored, the recipient's ABO type would be assigned, and type-specific products could then be issued.

Note that although it should be possible to determine the recipient's ABO type after receiving multiple uncrossmatched type O erythrocyte-containing products, the initial type and screen sample should be sent to the transfusion service as soon as possible after the patient arrives at the hospital and before a large quantity of these products are transfused to allow the transfusion service to quickly establish the recipient's ABO type. Knowing the recipient's ABO type gives the transfusion service the ability to switch recipients to ABO type-specific erythrocytes, thereby preserving the group O erythrocytes for those who can only receive this type of blood.[3]

The D typing of 15 of 695 (2.2%) patients in this study could not be determined on the first type and screen sample sent to the transfusion service during their resuscitation because they received both D+ and D− uncrossmatched erythrocyte-containing products before the sample was collected; in these cases mixed field agglutination was detected in their first type and screen, and it was not possible to determine whether they were D+ and were demonstrating mixed field agglutination because they received D− erythrocytes or vice versa. These patients were not included in the D analysis but were included in the forward and

reverse typing analysis. Given that many of the recipients of uncrossmatched erythrocytes are likely to be males, especially in civilian and military trauma situations,[4,5] the administration of D+ erythrocytes to a male recipient that later turns out to be D− is of little consequence.[5] In fact, it is worthwhile to consider the consequences of transfusing a D− female of childbearing potential with D+ erythrocyte-containing products; it is known that the rate of forming anti-D among hospitalized D− recipients of at least one unit of D+ erythrocytes is approximately 22%.[6–8] In addition, the probability that a Caucasian female might carry a D+ fetus is approximately 85%,[1] and the rate of fetal demise in a sensitized pregnancy is approximately 4%.[9] Thus, the overall rate of fetal demise in a D− female who receives D+ erythrocyte-containing products is approximately 1%, which should be considered when planning an erythrocyte resuscitation strategy. The potential for saving a bleeding woman's life with a D+ erythrocyte transfusion if D− erythrocytes are not available should be balanced with the small risk of adverse fetal consequences from D alloimmunization.

This study has several limitations. Factors that could perhaps have affected the agglutination strengths on the forward and/or reverse typings, such as the volume of fluids other than erythrocytes that might have been administered during the resuscitation, and cause of bleeding were not collected in this study because their effects on the agglutination strengths were likely to be less significant than the number of type O erythrocyte-containing products that were transfused. A statistical analysis of the changes in the median agglutination strengths on the forward and reverse typings between those who received the fewest number of uncrossmatched type O erythrocyte-containing products (*i.e.*, 1 unit) compared with those who received the greatest number of products (*i.e.*, more than 10 units) was not performed because most transfusion services require an agglutination strength of at least 2+ to establish the recipient's ABO group. Thus, the visual depiction of the median agglutination strengths (and 95% CI) in figures 3 and 5 reveals that for the vast majority of patients, the receipt of type O erythrocyte-containing products does not interfere with the determination of their ABO type. This was true for 665 of 695 (95.7%; 95% CI, 93.9 to 97.0%) of the recipients in this study, although the incidence of an invalid ABO type increased as the number of transfused products increased. Unless an automated instrument is utilized to perform the forward and reverse typings, the assignment of agglutination strengths is likely to be subjective and could vary between operators. However, given that the median agglutination strengths on the forward and reverse typings was either the maximum (4+) or nearly the maximum (3+) for the majority of the recipients of uncrossmatched type O erythrocyte-containing products, the subjectivity in determining the strength of agglutination would not likely affect the overall findings of this study. The detection of mixed field agglutination is also subjective because what might be considered a weaker agglutination strength by one technologist might be considered mixed field agglutination by another. Nevertheless, the data used in this study reflected real-world transfusion service practices, and the large number of recipients likely balanced the subjectivity of the agglutination strength interpretations. The results of the *in vitro* mixed field agglutination model should be interpreted with caution: it was not possible to determine how much blood had been lost by the actual patients in this study before their resuscitation with erythrocytes began; thus, determining the proportion of autologous and donor erythrocytes that were present when the mixed field agglutination was detected to compare with the dilutions in the model was not possible. Nevertheless, the *in vitro* model demonstrated similar trends to the patients in that as the proportion of added O+ erythrocytes, or for the patients the number of transfused O+ erythrocyte-containing products, increased so did the detection of mixed field agglutination. That the median agglutination strength for the forward D typing was always the maximum 4+, regardless of the number of uncrossmatched type O erythrocyte-containing products transfused, likely reflects the large number of D+ recipients receiving D+ products in this study. This probably also explains the relatively low frequency of detecting mixed field agglutination in the D typing. At one institution when mixed field agglutination was detected, the actual agglutination strength on the forward typing was not recorded. Thus, five patients with mixed field agglutination at this institution were excluded from this study, and so the overall rate of detecting mixed field agglutination in this patient population might be underestimated. Furthermore, the higher frequency of not being able to detect sufficiently strong agglutination on forward or reverse typing to establish the recipient's ABO type using the gel method compared with the frequency with which it had been used to test these patients requires further investigation in this setting. In addition, it is possible that not all eligible patients from each center were included in this study, thereby potentially introducing bias into these results. Last, a second sample (sometimes called a check type or confirmation sample) drawn from a recipient without a historical ABO type on file in the blood bank can be used for ABO verification and the detection of wrong-blood-in-tube errors. In this study agglutination was evaluated only in the first type and screen sample; therefore, wrong-blood-in-tube errors in this sample would not have been detected, and the agglutination strength results in the first type and screen sample could not be confirmed. Note that the decision to switch a non–group O patient who has received uncrossmatched group O erythrocytes to type-specific erythrocytes is complex and requires consideration of the number of group O units transfused as well as the quantity of incompatible plasma and platelets transfused.

The transfusion of up to 10 units, and potentially even higher quantities, of uncrossmatched type O

erythrocyte-containing products, which are potentially life-saving in massively bleeding patients, should not be withheld for fear of interfering with the subsequent determination of the recipient's ABO type. The ABO type of 30 of 695 (4.3%; 95% CI, 3.0 to 6.1%) of the recipients of uncrossmatched products in this study could not be determined from the first type and screen sample. Hospitals and emergency rescue services that are considering implementing prehospital transfusion strategies should not be dissuaded from implementing these programs because of concerns about complicating the interpretation of the recipient's immunohematology test results.

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## Competing Interests

The authors declare no competing interests.

## Correspondence

Address correspondence to Dr. Yazer: Vitalant, 3636 Boulevard of the Allies, Pittsburgh, Pennsylvania 15143. myazer@itxm.org. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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## Appendix. Members of the Biomedical Excellence for Safer Transfusion Collaborative

David McKenna, M.D.; David Stroncek, M.D.; Mark Fung, M.D.; Alan Tinmouth, M.D.; Dana Devine, Ph.D.; Rebecca Cardigan, Ph.D.; Ralph Vassallo, M.D.; Marc Germain, M.D.; JoAnna Reems, M.D.; Magali Fontaine, M.D.; Henk Garritsen, M.D.; Minoko Takanashi, M.D.; Richard Kaufman, M.D.; Simon Stanworth, M.D.; Meghan Delaney, M.D.; Alyssa Ziman, M.D.; José Cancelas, M.D.; Pieter van der Meer, Ph.D.; Denese Marks, M.D.; Claudia Cohn, M.D.; Beth Shaz, M.D.; Pierre Tiberghien, M.D.; Mindy Goldman, M.D.; John Girdlestone, M.D.; Shibani Pati, M.D.; Richard Schäfer, M.D.; Joseph (Yossi) Schwartz, M.D.; Nancy Dunbar, M.D.; Mark Yazer, M.D.; Monica Pagano, M.D.; Cynthia So-Osman, M.D.; Femke Noorman, M.D.; Torunn Oveland Apelseth, M.D.; Tamir Kanias, Ph.D.; Moritz Stolla, M.D.; Emanuele di Angelantonio, M.D.; Katja van den Hurk, M.D.; Whitney R. Steele, M.D.; Christian Erikstrup, M.D.; Eva-Maria Merz, M.D.