Report

Red blood cell phenotyping after transfusion: an in vitro model

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Recipient red blood cell (RBC) phenotyping using serologic techniques, within 3 months of a transfusion, is considered unreliable. We conducted in vitro experiments to determine how long recipient RBC phenotyping results would be compromised after an allogeneic transfusion. In vitro models were created to mimic in vivo posttransfusion ratios of "transfused" RBCs with either a single or a double dose of an antigen and "autologous" RBCs negative for the corresponding antigen at 10-day intervals from day 0 to day 90 in hypothetical recipients with varying weights and hematocrits (Hct) receiving varying numbers of RBC units. In general, a reliable recipient RBC phenotype was possible earlier after transfusion in larger recipients, those with higher Hct, and those transfused with fewer RBC units and if the transfused units had the antigen of interest in single, rather than double, dose. We believe that a reliable RBC phenotype, using routine serologic techniques, can often be obtained well before 3 months after transfusion. Similar studies with other donors, antigens, antisera, and methods and in actual patients will be useful. ***Immunohematology* 2013;29:93–6.**

Key Words: RBC, phenotyping, posttransfusion, in vitro model

Recipient red blood cell (RBC) phenotyping may be useful in determining what clinically significant alloantibodies a recipient could make by identifying the antigens that the recipient lacks. It has been considered difficult to do this after transfusion, but it is the regularly transfused patient in whom this information is most useful. Alternatives exist—e.g., genotyping—but this may not be possible everywhere. We used an experimental model to determine how soon after a hypothetical "transfusion" an unequivocal "recipient" RBC phenotype could be determined using conventional serologic methods.

Materials and Methods

In vitro mixtures of RBCs were created to mimic in vivo "posttransfusion" ratios of "autologous" to "transfused" RBCs at 10-day intervals from day 0 to day 90 after transfusion of varying numbers of RBC units in recipients of varying weights and with varying degrees of anemia.

In our model, C+, Jk(a+), or K+ RBC units were transfused to C–, Jk(a–), or K– recipients, respectively. RBC transfusions with a single or double dose of the antigen concerned were considered separately. The transfused and autologous RBCs used in the experiments came from four healthy blood donors or volunteers (blood groups: O, rr, K–k+, Jk[a–b+]; O, R_1R_1, K–k+, Jk[a+b–]; O, R_1r, K+k–, Jk[a–b+]; O, R_1r, K+k+, Jk[a+b+]) whose units were less than 1 week old.

Nominal male recipients weighing 50, 70, or 100 kg, with estimated blood volumes (EBV) appropriate to weight, with either of two levels of anemia (hematocrit [Hct] 25% or 15%) were considered. The EBV was taken as 70 mL/kg, i.e., the midpoint of the normal range in either sex (60–80 mL/kg).[1] The volume of autologous RBCs in the recipient was calculated from the EBV and Hct. Recipients with Hct of 25 percent were considered to have received one, two, or three units of packed RBCs and those with Hct of 15 percent, two, three, or four units.

Per the standard specifications for leukocyte-reduced RBC units in New Zealand, the average volume of RBC units was determined to be 300 mL, the average Hct was 60 percent, and posttransfusion RBC recovery (PTR) was 80 percent, resulting in an RBC volume per RBC unit transfused of 144 mL. It was assumed that transfused RBCs survived optimally. Thus, the loss of transfused RBCs per day was taken as 1/120 of the RBC volume transfused on day 0 (144 mL × number of RBC units transfused).

For instance, a 50-kg recipient with an EBV of 3500 mL (70 mL/kg) and Hct of 15 percent might have an RBC volume of approximately 525 mL. If such a recipient were to be transfused with three RBC units, the volume of transfused RBCs on day 0 would be 144 mL × 3 or 432 mL. Assuming that 1/120 of the transfused RBCs are destroyed each day, surviving transfused RBCs after 10 days would be 396 mL, after 20 days would be 363 mL, and so on. Similar calculations were done for all nominal recipients. Note that it was assumed that the autologous RBC volume stayed constant as a result of balanced production and destruction. Posttransfusion ratios of autologous to transfused RBCs at different times were calculated for all recipients, factoring in recipient weight, Hct, and days after transfusion as described previously. These are shown in Figure 1 expressed as calculated percentages of RBCs in the recipient that are likely to be transfused RBCs.

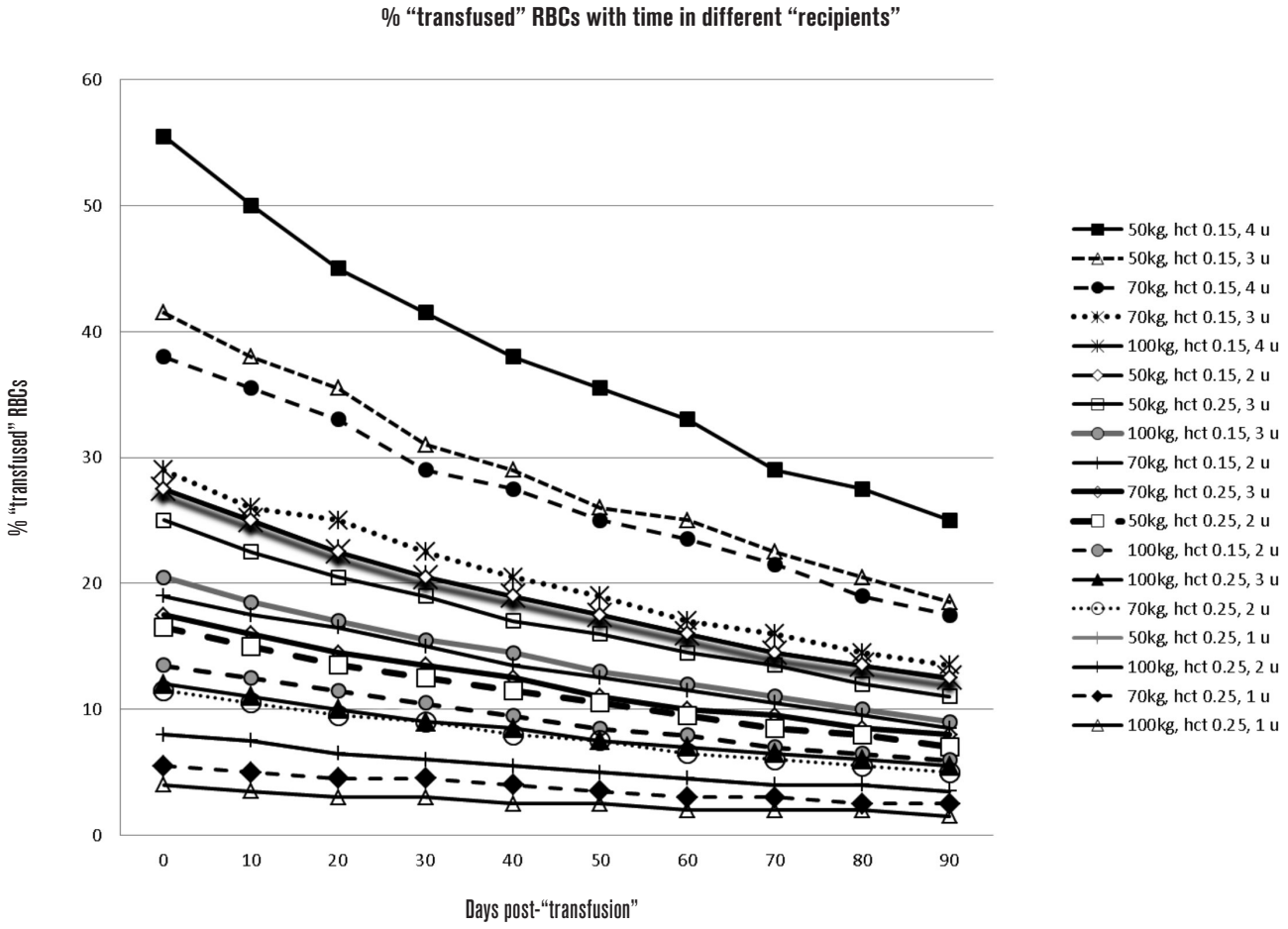

Fig. 1 Calculated percentage of transfused red blood cells (RBCs) remaining at varying times after transfusion in recipients with different values for weight, hematocrit (Hct), and number of RBC units transfused.

The actual volumes of the RBC suspensions—either a 3 percent or 0.8 percent suspension of the RBC mixture (Ortho Biovue and DiaMed-ID, respectively—see later section)—used varied, from the one with the highest autologous-to-transfused ratio (1750 µL:65.8 µL in the nominal 100-kg patient with an Hct of 25%, at day 90 after transfusion of one RBC unit) to the one with the lowest autologous-to-transfused ratio (576 µL:525 µL in the nominal 50-kg patient with an Hct of 15%, at day 0 after transfusion of four RBC units). In each case we started with the autologous-to-transfused RBC ratio most likely to show a clear negative reaction (a score of 0), i.e., a high ratio. Then, mixtures with successively lower autologous-to-transfused RBC ratios were tested until the point at which a clear negative reaction was no longer obtainable. The last ratio giving a clear negative reaction was taken as the day beyond which it was possible to ascertain the autologous, antigen-negative RBC phenotype. This was done separately for transfused RBCs with a single or double antigen dose.

RBC phenotyping was done using column agglutination methods (Ortho BioVue System, Ortho Clinical Diagnostics, Raritan, NJ) for C and K and (DiaMed-ID Micro Typing System, DiaMed AG, Morat, Switzerland) for Jk^a. Commercially obtained antisera (anti-C and anti-Jk^a, Lorne Laboratories, Reading, UK) were used. The anti-K was incorporated in the gel card (Ortho BioVue System, Ortho Clinical Diagnostics). Validated New Zealand Blood Service procedures were followed. Positive and negative controls were run in parallel with test samples.

Results

All four variables studied (recipient weight and Hct, the number of antigen-positive RBC units transfused, and the dose of the relevant antigens in the transfused units) affected which day after transfusion the autologous, antigen-negative RBC phenotype could be determined (Table 1).

Table 1. Determination of autologous, antigen-negative red blood cell phenotype*

Patient weight (kg)	Hct (%)	Number of units transfused	Phenotype of transfused RBCs					
			C+c−	C+c+	Jk(a+b−)	Jk(a+b+)	K+k−	K+k+
50	15	4	—	—	—	—	—	—
50	15	3	—	—	—	—	—	—
70	15	4	—	—	—	—	—	—
70	15	3	—	—	—	—	—	—
100	15	4	—	—	—	—	—	—
50	15	2	—	—	—	—	—	—
50	25	3	—	—	—	—	—	80
100	15	3	—	—	—	—	—	—
70	15	2	—	—	—	—	—	—
70	25	3	—	—	—	—	—	40
50	25	2	—	—	—	—	—	40
100	15	2	—	90	—	90	—	90
100	25	3	—	80	—	80	—	0
70	25	2	—	80	—	80	90	0
50	25	1	70	40	90	40	90	50
100	25	2	70	40	90	40	50	0
70	25	1	30	0	50	0	10	0
100	25	1	0	0	10	0	0	0

*Calculated number of days after "transfusion" of C+, Jk(a+), or K+ (single or double antigen dose) red blood cells (RBCs) when an antigen-negative phenotype can be (**shaded numbers**) or cannot be (**dashes**) established serologically before 3 months after transfusion in an unequivocal recipient, with consideration of recipient weight, hematocrit (Hct), and number of RBC units "transfused" and with different ratios of antigen-positive ("transfused") and antigen-negative ("autologous") RBCs corresponding to different 10-day periods after transfusion.

In general, in nominal recipients with an Hct of 25 percent, we were able to determine the autologous RBC phenotype relatively early after transfusion. This was especially true of larger recipients; of those transfused with fewer RBC units; and if the RBCs expressed a single, rather than a double, dose of the antigen concerned. In nominal recipients with a Hct of 15 percent weighing 50 or 70 kg, irrespective of the antigen, its dose, or the number of RBC units transfused, the autologous, antigen-negative phenotype could not be unequivocally determined even at day 90 after transfusion. This was also largely true for the 100-kg recipient with an Hct of 15 percent except that, in this case, a clear negative (autologous) phenotype could be established in the day 90 sample with two transfused RBC units if the C, Jk^a, or K antigen was present in a single dose (Table 1).

Discussion

Recipient RBC phenotyping may be useful in at least three situations in recently transfused patients: first, to avoid further exposure to clinically significant RBC antigens in those likely to require RBC transfusions long-term (ideally such recipients should be phenotyped before the first transfusion); second, to corroborate alloantibody specificities; and third, to allow alloadsorption studies using phenotype-matched RBCs in patients with autoimmune hemolytic anemia to be more easily performed.

Serologic determination of RBC phenotype in recently transfused patients is considered difficult[2] and is generally not recommended within 3 months of a transfusion.[3] Alternatives in this situation include using the resistance of RBCs from patients with sickle cell disease, relative to transfused RBCs, to hypotonic lysis[4]; autologous reticulocyte phenotyping after initial separation in microhematocrit tubes[5] or by using immunomagnetic[6] or color flow cytometric methods[7]; and genotyping using polymerase chain reaction and related methods.[8] Many such methods are not generally available.

The 3-month period referred to previously does not take into account variables such as recipient weight and Hct, the number of transfused units, or the antigen dose in the transfused units—the effects of all of which were examined in this in vitro study. Patient weight determines, to an extent, the blood volume, which is the volume of dilution for transfused cellular components. If the volume of RBCs transfused is high relative to the patient's volume of dilution and Hct, one might expect a relative delay in establishing an unequivocal patient RBC phenotype after transfusion. We might also expect similar delays in patients with chronic RBC synthesis failure or hemolysis as opposed to, say, otherwise well patients who are anemic after an acute blood loss in whom it might be expected that autologous RBC synthesis would be significantly increased after the acute event.

To our knowledge, an in vitro model of this sort has not previously been described in the literature. Our experiments suggest that, at least for the antigens we considered, a clear RBC phenotype can be obtained for some transfusion recipients well before 3 months after transfusion, using routine serologic methods, although this may not be possible in others, for instance, small-sized individuals with low Hct given relatively large RBC transfusions (Table 1). We expect that similar results will be obtained with other RBC antigens.

Both the Council of Europe and the US Food and Drug Administration guidelines[9,10] require a PTR of at least 75 percent 24 hours after transfusion. We assumed a PTR of 80 percent, which is within the range that might be expected for transfused RBCs toward the end of shelf-life,[11] and we used this figure in our experiments to determine transfused-to-autologous RBC ratios at various time intervals. Of course,

higher recoveries (e.g., approximately 90%) may be obtained with fresher units,[11] and this would tend to increase the proportion of transfused RBCs relative to autologous RBCs at various times after transfusion. However, Luten et al.[11] also noted that PTR in transfused patients may be lower than in healthy volunteers, which would have the opposite effect, i.e., to decrease the proportion of transfused RBCs relative to autologous RBCs.

In most patients (again, with the exception of individuals with low EBV and low Hct given relatively large RBC transfusions), the proportion of antigen-positive, transfused RBCs is likely to be small relative to that of autologous RBCs. We considered only unequivocal negative results as indicative of the true autologous, antigen-negative RBC phenotype. However, in the context of RBC phenotyping in recently transfused patients, the relevant questions are, is there an antigen-negative RBC population, and, if so, could this be of recipient origin, taking into consideration the patient's weight and Hct, the number of RBC units transfused, and the time after transfusion? Furthermore, mixed populations of RBCs are easily distinguishable with gel agglutination methods.

When considering patient RBC phenotyping after transfusion, these factors should be taken into account. Information on antigen doses in transfused units will usually be unavailable; however, in many instances and with many of the clinically significant RBC antigens, RBCs are more likely to have a single rather than double dose of the antigen. Studies with other antigens, test systems, and antisera, as well as in real patients, will be useful.

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