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In Premature Infants There is No Decrease in 24-h Post-Transfusion Allogeneic Red Cell Recovery After 42 Days of Storage

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Abstract

Background—Critically ill preterm very low birth weight (VLBW) neonates (birthweight < 1.5 kg) frequently develop anemia that is treated with red blood cell (RBC) transfusions. Although RBC transfused to adults demonstrate progressive decreases in post-transfusion 24-hour RBC recovery (PTR₂₄) during storage—to a mean of about 85% of the FDA allowed 42-day storage—limited data in infants indicate no decrease in PTR₂₄ with storage.

Study Design and Methods—We hypothesized that PTR₂₄ of allogeneic RBCs transfused to anemic VLBW newborns: 1) will be greater than PTR₂₄ of autologous RBCs transfused into healthy adults; and 2) will not decrease with increasing storage duration. RBCs were stored at 4°C for 42 days in AS-3 or AS-5. PTR₂₄ was determined in 46 VLBW neonates using biotin-labeled RBC and in 76 healthy adults using ⁵¹Cr-labeled RBC. Linear mixed model analysis was used to estimate slopes and intercepts of PTR₂₄ versus duration of RBC storage.

Results—For VLBW newborns, the estimated slope of PTR₂₄ versus storage did not decrease with the duration of storage (P=0.18) while for adults it did (P<0.0001). These estimated slopes

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differed significantly in adults compared to newborns ($P=0.04$). At the allowed 42 d storage limit, projected mean neonatal PTR_{24} was 95.9%; for adults, it was 83.8% ($P=0.0002$).

Conclusions—These data provide evidence that storage duration of allogeneic RBCs intended for neonates can be increased without affecting PTR_{24} . This conclusion supports the practice of transfusing RBCs stored up to 42 days for small-volume neonatal transfusions to limit donor exposure.

Keywords

RBC storage media; RBC storage lesion

INTRODUCTION

During the first few weeks of life when illness and the resultant laboratory testing are most intense, preterm very low birth weight (VLBW) newborn infants weighing <1.5 kg at birth commonly receive allogeneic RBC transfusions. To ensure optimal efficacy and avoid decreased survival of transfused RBCs and to thereby limit the number of RBC transfusions, it is important that allogeneic RBC units stored in the blood bank must be high quality. When RBCs stored for 42 d in extended storage media (e.g., AS-1, AS-3, AS-5, and AS-7) are transfused to adults, an average of 85% of the transfused RBCs remain in the recipient's circulation after 24-h.¹ In the only prior study of 24-h RBC post-transfusion recovery (PTR_{24}) in newborn infants,² PTR_{24} for 11 VLBW neonates transfused with RBCs stored in AS-3 media did not decrease with increasing duration of storage; PTR_{24} was 98.6% for storage ≤ 21 days and 96.9% for storage >21 days.

For FDA licensing of storage media, the assessment of PTR_{24} is required. For this purpose, measurements have been performed in healthy adults using autologous RBCs labeled with the radioactive isotope 51-chromium (^{51}Cr).¹ For licensing of storage media, PTR_{24} testing is not required in other patient populations, such as infants and children. Although ^{51}Cr has been used exclusively for FDA licensing, radiation exposure of infants, children, and pregnant women is deemed ethically unacceptable in the US.³ In Europe, similar concerns about radiation exposure have been extended to adults. Consequently, the European standards for licensing approvals of RBC transfusion commercial devices do not include a requirement for PTR_{24} determination.^{4,5}

No prior RBC kinetic studies have performed direct statistical comparisons of PTR_{24} in infants and adults. The present study offers this comparison by utilizing PTR_{24} data from three previously reported neonatal RBC kinetic studies^{2,6,7} and from four adult RBC studies.^{8–11} Doing so is desirable for comparing PTR_{24} results in two such markedly different subject populations with their different RBC circulatory environments.¹² The different circulatory environments have been demonstrated in studies reporting markedly different long-term RBC survival in newborns and in adults. Specifically, adult RBCs survive approximately half as long in the circulation of infants as in adults.⁷ The opposite has also been demonstrated: fetal RBCs from infants delivering at term survive approximately twice as long when in the adult circulation as they do in newborn infants.^{13,14}

Labeling of RBC surface proteins with biotin (Vitamin B7), followed by flow cytometric enumeration of the biotinylated RBCs (BioRBCs) remaining in circulation, offers an alternative method that avoids exposure of transfused subjects to radioactivity.¹⁵ Employing the biotin method also adds two additional important capabilities compared to ⁵¹Cr method: 1) the ability to label RBCs at multiple, discrete biotin densities (e.g., to compare effects of storage media on RBC survival); this approach allows for simultaneous quantification of red cell volume and survival in the same subject; and 2) the ability to accurately tracking BioRBC survival for at least 95% of the RBC lifespan. The latter is not possible with ⁵¹Cr labeled RBC because of elution of the chromium that varies unpredictably between individuals from 0.5% to 2.5% per day, thus limiting quantification of RBC survival to 28 day.¹⁶

Although most adult studies were done with ⁵¹Cr and the sole neonatal study was done with BioRBC, we infer that the reported difference between PTR₂₄ for adults and neonates is not likely attributable to differences in the labeling methods because the two methods yield similar results when employed simultaneously. In healthy adults in which ⁵¹Cr- and biotin-labeled autologous RBCs were concurrently administered, no differences in 24-h RBC post-transfusion recovery or in long-term RBC survival were demonstrated.^{11,17} Accordingly, we hypothesize that PTR₂₄ of allogeneic RBCs administered to anemic, preterm VLBW newborns is greater than PTR₂₄ of autologous ⁵¹Cr-labeled RBCs transfused into adults. Further, we hypothesize that PTR₂₄ in neonates will not decrease with increasing duration of RBC storage. To test these hypotheses, we retrospectively compared PTR₂₄ data from three previously reported neonatal studies with PTR₂₄ data from four PTR₂₄ adult studies.^{2,6–11}

MATERIALS AND METHODS

The studies received IRB approval from both study sites, the University of Iowa and the University of Cincinnati. Written informed consent was required and obtained from all adult study subjects and from the parents of study infants.

Study subjects

Neonatal subjects included a convenience sample of 46 anemic infants with an estimated gestational age at birth of 31 weeks who were being ventilated for respiratory distress and anticipated to survive. All neonates were anemic and receiving clinically indicated allogeneic adult donor RBC transfusions as reported previously.^{2,6,7} Infants with major congenital anomalies were excluded.

Adults included healthy individuals meeting criteria as blood donors. All had been enrolled in previously reported RBC kinetic research studies in which autologous RBCs labeled with ⁵¹Cr were reinfused.^{8–11}

Neonatal and adult study protocols

Neonatal subjects received allogeneic BioRBCs that had been leukoreduced (Leukotrap RC System; Medsep, Covina, California) and stored up to the 42-day FDA limit while maintained in the blood bank per hospital protocol in AS-3 RBC storage media (Nutricel, Medsep Corporation, formerly Cutter Biologicals) prior to biotin labeling as described

below. Actual storage time depended on blood compatibility and availability and varied from 2 to 42 days. On the day that a clinical transfusion was ordered, aliquots were taken from blood bank RBC units pre-assigned to each infant, irradiated (3000 cGy) on the same day as the RBC transfusion, and administered according to standard transfusion practice for neonates at our institution, i.e., as 15 mL/kg of packed RBC at hematocrits of $85 \pm 2\%$. The first 11 to 14 mL/kg of donor RBCs were transfused as a routine clinical transfusion over 3 to 4 hr; the remaining 1 to 4 mL/kg were labeled with biotin at one to four separate BioRBC densities and then mixed and transfused in under 15 minutes through an 18 μ m filter to remove microaggregates (Hemo-Nate; Utah Medical Products, Midvale, UT 84047) immediately following the conclusion of the clinical transfusion.

Adult study subjects donated 500 mL of blood which was stored in AS-3 or AS-5 RBC storage media (Optisol™, Terumo BCT Inc., Lakewood, CO 80215) after undergoing in-line leukoreduction filtration (Terumo BCT, Inc.). These autologous RBC units were stored in the blood bank for 0, 21, 35, or 42 days prior to re-administration; approximately 10 mL of RBCs were labeled with ^{51}Cr and reinfused at a rate of 5 to 7 mL/min.

Laboratory methods

Biotin labeling of neonatal RBCs administered to neonates—Adult donor allogeneic RBCs administered to neonates were biotinylated as previously described.¹⁸ Briefly, RBCs were washed using a carbonate-buffered dextrose sodium phosphate wash solution to remove plasma and resuspended at a hematocrit of 25%. Hematocrit adjustment was verified using the Sysmex XE-2100 (Sysmex, Kobe, Japan). Subsequently, RBCs were biotinylated using the biotin 3-sulfo-N-hydroxysuccinimide ester (EZ-LINK Sulfo-NHS-Biotin; Pierce Chemical, Rockford, IL 61101). Depending on the primary objectives of the original study, up to four separate concentrations ranging from 6 to 162 μ g of sNHS-biotin per mL of packed RBCs were chosen. These choices produce discreet biotin labeling densities that can be individually tracked for at least 95% of the RBC lifetime. Minutes prior to transfusion, a 0.5 mL sample of the BioRBC mixture was taken for bacterial culture.

BioRBC Flow cytometric determination—After infusion, the number of BioRBCs for each of the different BioRBC populations was determined by flow cytometry and expressed as a fraction of the total number of RBCs counted. Flow cytometric measurements were performed in triplicate on 10 μ L of leftover whole blood salvaged from laboratory blood testing as described previously.¹⁸ For each sample, 1×10^6 RBCs were counted.¹⁹ For the rare samples exhibiting overlap in the fluorescence histogram between the BioRBC peaks or between the unlabeled RBC and the lowest biotin density, mixed modeling was used to calculate the proportion of RBCs in each population as previously described.²⁰ The lower limit of quantitation for individual BioRBC density peaks is 0.06% of total RBCs.²¹

For BioRBCs, PTR_{24} was calculated as the ratio of the proportion of each population of BioRBCs remaining in the circulation at approximately 24 h (15–36 hr per study protocol) after infusion of the BioRBCs relative to the proportion of the same population of BioRBCs in circulation at the first available time point (usually 10 to 180 min post-BioRBC transfusion, but occasionally as late as to 180 min post-transfusion per study protocol).

⁵¹Cr labeling of adult RBCs—On the day of reinfusion, each study RBC unit was inspected for signs of hemolysis, clotting, and discoloration, and the bacterial culture was taken from each unit 5 days before was confirmed to be negative. The unit was then mixed by hand and an aliquot of the RBCs was collected from the unit and labeled with 370 to 740 kBq of ⁵¹Cr using standard techniques over approximately 90 min as follows.²² The labeling agent, sodium 51-chromate, was mixed aseptically with the aliquot of RBCs at room temperature for 30 minutes followed by a single wash step to remove free ⁵¹Cr label. An aliquot of the final ⁵¹Cr labeled RBC mixture was saved as a radioactivity recovery standard. Approximately 4 mL of the ⁵¹Cr-labeled RBCs were drawn up into a syringe and infused.

Determination of radioactivity in blood containing ⁵¹Cr-labeled RBCs—The amount of RBCs and total radioactivity infused were determined using the method of Moroff et al.²² as recommended by the International Committee for Standardization in Hematology.¹⁶ The infusion of the radiolabeled aliquot and all subsequent blood sampling was carried out according to study site standard operating procedures and as described by Moroff et al.²² Briefly, after infusion of approximately 10 mL of stored ⁵¹Cr RBCs, seven blood samples were drawn over 30 minutes immediately after completion of the RBC infusion. An additional sample was drawn at 24 hr. The ⁵¹Cr specific activity of the samples was quantitated by gamma counting and ⁵¹Cr was calculated using ⁵¹Cr specific activity after correcting for radioactive decay and for the loss of ⁵¹Cr label over time using an elution multiplicative factor.²³ PTR₂₄ was calculated as the ratio of the radioactivity per mL of whole blood in the 24-h sample to the radioactivity per mL of whole blood extrapolated to time 0 from the first seven samples. All ⁵¹Cr samples were counted at the same time.

For ⁵¹Cr-labeled RBCs, time zero was determined by calculating the ⁵¹Cr counts (adjusted for Hct) for time points 5, 7.5, 10, 12.5, 15, and 20 min and extrapolated using the natural logarithm of the regression line of these values to time zero. The PTR₂₄ was determined by dividing the 24-hr time point counts by the time zero counts.²³

Statistical methods—Linear mixed model analysis was used to examine the relationship between PTR₂₄ and RBC storage time in newborns and in adults. This method of analysis properly accounts for subjects who had more than one data point. From this analysis, estimates of the slopes were obtained for newborns and for adults. The null hypothesis was that there was no effect of storage time on PTR₂₄; H₀: slope=0. For each group, we also tested for a difference in slope between the groups. To assess for potential effects of neonatal demographic factors (i.e., sex, birth weight, gestational age, weight at study, and age at study) on the relationship between PTR₂₄ and RBC storage time linear mixed model analysis was performed on the newborn data. To assess potential effects of sex and age, similar analysis was performed on the adult data. All statistical analyses were performed using SAS (version 9.4). A *P*-value of 0.05 was considered statistically significant.

RESULTS

Demographics

For the 46 neonates mean (±SD) birth weight and gestational age were 907 ± 254 g and 27.1 ± 1.8 wk, respectively. There were 21 (46%) males and 8 (17%) twins; the rest were

singletons. Four of the 46 (8%) were small for gestational age (<2SD for weight adjusted for gender, multiple birth, and gestational age at birth).²⁴ As per inclusion criteria, all received mechanical ventilation within the first day of life. Mean post-natal age at the time of the BioRBC transfusion was 16 ± 16 d (range: 0 to 57 d). The mean blood bank storage time of the RBC transfused to the neonates was 12 ± 9 d (range: 2 to 42 d). The mean post-transfusion time of the blood sampling used in calculating PTR₂₄ were 29 ± 34 minutes (range: 10 to 200 min) and 24 ± 4 hr (range: 15 to 36 hr), respectively.

The mean age of the 76 healthy adults, 33 of which were females, was 44 ± 12 yr.

Comparison of adult PTR₂₄ data for concurrent biotin- and ⁵¹Cr-labeled RBC

Because our neonatal study used biotin labeled RBCs and the adult study used ⁵¹Cr-labeled RBCs, we evaluated a potential method effect. Two studies of healthy adults compared biotin method to the ⁵¹Cr method.^{11,17} In both, freshly drawn aliquots of autologous RBCs were labeled with either biotin or ⁵¹Cr, mixed, and concurrently re-infused. Because the labeled RBCs were reinfused within 8 h, these are denoted as “zero” storage times.^{11,17} For the 10 healthy adults in the first study, there was no difference in mean PTR₂₄ between the biotin- and ⁵¹Cr-labeled RBCs.¹¹ PTR₂₄ was 99.9 (95% CI: 94.3%, 105.5%) and 99.4% (93.8%, 105.0%), respectively, $P = 0.84$.¹¹ To further evaluate a potential method effect on PTR₂₄, PTR₂₄ was quantitated by both methods in an additional 10 healthy adults (4 repeat participants) under conditions of accelerated removal of the RBCs. Participants were selected for Rh-D+ antigen on RBC, and their autologous Rh-D+ RBCs were coated *ex vivo* with either low or high concentrations of anti-D antibody immediately prior to reinfusion; low concentration of anti-D antibody did not accelerate RBC removal but high concentration did.¹⁷ When linear mixed model analysis was applied to combined data for all 20 RBC PTR₂₄ determinations, the mean PTR₂₄ for biotin- and ⁵¹Cr-labeled RBCs were also not different: 95.0% (90.9, 99.0%) and 94.2% (90.2, 98.3%), respectively (mean difference of 0.8; 95% CI: -4.3%, 5.8%; $P = 0.76$).

Comparison of neonatal and adult PTR₂₄ relative to the duration of storage

The developmental difference in storage effect on PTR₂₄ was compared between infants and adults by characterizing the dependence on storage duration for each group. For the infant data, we initially performed linear mixed model analysis to evaluate the possibility that there might be an effect of BioRBC density on the relationship between PTR₂₄ and RBC storage duration. No such effect was detected as judged by finding no difference in slopes among the three tiers of densities ($P = 0.42$); accordingly, our final slope comparison pooled the data from the three BioRBC tiers of densities. There was a statistically significant difference in the time zero y-axis intercepts of the three BioRBC density tiers: High (mean and CI) = 99.7% (96.5, 102.9%), Medium = 101.4% (98.4, 104.3%), and Low = 102.1% (99.2, 105.0%). Because these all were not significantly different from 100%, we infer that these differences are of no physiological relevance.

The finding of no difference in slopes mandated adoption of a model that had a common slope but significantly different intercepts among the three tiers of densities ($P < 0.001$). For all subsequent analyses, the model with a common slope but different intercepts for the three

BioRBC densities was used. Applying this linear mixed model to the combined newborn and adult data, indicated that there was no significant decline in PTR₂₄ with increasing storage duration in newborns; slope = 0.13% per d (95% CI: -0.32, 0.06; P=0.18). In contrast, the ⁵¹Cr-labeled RBCs for the 76 adults demonstrated a significant decline in PTR₂₄ with storage, i.e., decreasing slope: -0.34% per d (95% CI: -0.43, -0.25; P<0.0001). The rate of PTR₂₄ decline with storage time for adults differed significantly from that of the newborns (P=0.04) (Figure 1).

From this fitted mixed model based on the combined newborn and adult data, an estimate of the mean PTR₂₄ at 42-d of RBC storage (the maximum duration allowed for AS-3 and AS-5 by the FDA) was then computed for allogeneic RBCs administered to adult study subjects and to a single, combined group of newborns. For adults, mean PTR₂₄ at 42-d of RBC storage was 83.8% (95% CI: 81.9, 85.7%). This is consistent with previous reports.¹ In contrast, for the combined newborn group the estimated mean PTR₂₄ at 42-d RBC storage of 95.9% (95% CI: 90.1%, 101.8%) was significantly higher (P=0.0002).

We investigated the generalizability of these observations to other infants by examining the effects of demographic factors. None of the infant variables (sex P=0.55; gestational age at birth P=0.82; birth weight P=0.84; weight at study P=0.99; and postnatal age at study P=0.56) had a significant effect on the slope of PTR₂₄ versus duration of storage by linear mixed modeling. Likewise, there was also no significant effect of sex (P=0.85) or age (P=0.33) on the slope of PTR₂₄ in adults.

DISCUSSION

The present data using extended RBC storage media demonstrate striking differences in the 24-h recovery of transfused RBCs in newborns compared to adults. Unlike PTR₂₄ of allogeneic RBCs transfused to adults, allogeneic RBCs transfused to newborn infants showed no decrease in PTR₂₄ with increasing duration of RBC storage. Moreover, when the newborn and adult slopes for the plots of PTR₂₄ versus duration of RBC storage were compared, the two differed significantly. Because decreased survival of transfused RBC lessens the effect of the transfusion on correcting anemia and thus increases the need for subsequent RBC transfusions, RBC survival is import.

Ethical considerations dictated two additional factors that differed between the neonatal and adult data sets: 1) biotin was the label for newborns, and ⁵¹Cr for adults; and 2) the source of the RBCs differed: allogeneic RBCs for newborns, and autologous RBCs for adults. Could the difference in PTR₂₄ observed here reasonably be attributed to either of these two factors? Examination of other studies suggests not. In transfusions of autologous RBCs labeled with biotin or ⁵¹Cr, no PTR₂₄ differences have been observed. Moreover, autologous RBCs would be expected to be more compatible than allogeneic RBCs and to thus have higher PTR₂₄ values compared to allogeneic RBCs. Thus, the source difference would have masked a neonatal-adult difference, not produced one making source difference an explanation for the differences observed. We therefore contend there is no sound basis for concluding that these two methodological differences negate the main findings of this study.

The precise mechanisms responsible for the differences observed in the newborn and adult PTR₂₄ results are currently unknown. However, previous observations suggest a likely role for the immune system because of the comparative immaturity of the VLBW infants' immune system versus adults. Indeed, substantial attenuation of acquired immunity in newborn infants has long been recognized.²⁵ This developmental difference is the primary basis for the American Association of Blood Banks recommendation that infants receiving RBC transfusions require pre-transfusion antibody screening only once in the first four months of life, providing the initial result is negative.²⁶ The comparative function of the innate immune system (i.e., tissue macrophages and related cells such as Kupffer cells), likewise, must be considered, as these cells are responsible for the rapid and efficient removal of senescent RBCs and RBCs that have sustained post-transfusion "storage lesions."²⁷ In the present report, macrophages of newborns seem to have diminished ability to remove RBCs sustaining *ex vivo* storage lesion injury. Future studies defining how macrophages from infants and adults respond to different "eat-me signals" in their central role in controlling apoptotic programmed cell death will be important. Thus, based on the present findings, we speculate that neonatal macrophages respond differently to these RBCs with storage lesions than adult macrophages, thus reflecting their immaturity. Key recognition, engulfment, and degradation molecules associated with normal functioning of adult macrophages include CD47, CD200, phosphatidylserine, thrombospondin-1, and likely other factors. These capacities may be ineffective—or missing—in newborn infant macrophages as a result of their developmental immaturity.^{28,29}

Notable limitations in the present study include small number of infant PTR₂₄ measurements for RBCs stored longer than three weeks. Moreover, the sample sizes for both newborns ($n=46$) and adults ($n=76$) are relatively modest. Although PTR₂₄ data for 11 of the newborn infants included in this study have been reported previously,² the present study expands these data by more than three-fold. We also acknowledge that in this analysis of PTR₂₄ among groups of study subjects derived from several previously published studies, institution-specific factors beyond our control may have affected our findings and thus be considered a limitation. Specifically, due to maldistribution of neonates and adults at the two study sites, it was not possible to perform a sub-analysis of adults versus VLBW neonates within a single institution. A final study limitation is that the adult allogeneic RBC administered to neonates were irradiated while that the adult autologous RBC administered to adults were not. This seems unlikely to be an important limitation since in adults, PTR₂₄ of RBC irradiated two days prior to transfusion demonstrated no significant decline in PTR₂₄ while a 14 d interval demonstrated a significant decline.³⁰

In summary, observations presented here provide evidence of a significant effect of storage duration on PTR₂₄ in adults, but not newborn infants. These observations support the practice to transfuse RBCs, stored throughout the currently permitted FDA 42 d storage limit for RBCs, to infants receiving small-volume transfusions during the anemia of prematurity—rather than limiting storage to fewer days. Also, given the moratorium on radiation exposure to children in research, the biotin label seems to be the best alternative to radioactive ⁵¹Cr for RBC kinetic studies in children.¹⁵

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References

1. Hess JR, Collaborative BEfST. Scientific problems in the regulation of red blood cell products. *Transfusion*. 2012; 52:1827–35. [PubMed: 22229278]
2. Strauss RG, Mock DM, Widness JA, Johnson K, Cress G, Schmidt RL. Posttransfusion 24-hour recovery and subsequent survival of allogeneic red blood cells in the bloodstream of newborn infants. *Transfusion*. 2004; 44:871–6. [PubMed: 15157254]
3. Diekema DS. Conducting ethical research in pediatrics: a brief historical overview and review of pediatric regulations. *J Pediatr*. 2006; 149:S3–11. [PubMed: 16829239]
4. Guidelines for the Blood Transfusion Services in the United Kingdom. The Stationary Office; Norwich, U.K.: 2013.
5. Council of Europe. Guide to the preparation, use and quality assurance of blood components: recommendation No R (95) 15. Strasbourg: Council of Europe Pub; 2015.
6. Nalbant D, Bhandary P, Matthews NI, Schmidt RL, Bogusiewicz A, Cress GA, Zimmerman MB, Strauss RG, Mock DM, Widness JA. Comparison of multiple red cell volume methods performed concurrently in premature infants following allogeneic transfusion. *Pediatr Res*. 2013; 74:592–600. [PubMed: 24088873]
7. Widness JA, KDJ, Mock DM, Matthews NI, Nalbant D, Cress GA, Schmidt RL, Strauss RG, Zimmerman MB, Veng-Pedersen P. Autologous infant and allogeneic adult red cells demonstrate similar concurrent post-transfusion survival in very low birth weight neonates. *J Pediatr*. 2015
8. Cancelas JA, Dumont LJ, Rugg N, Szczepiorkowski ZM, Herschel L, Siegel A, Pratt PG, Worsham DN, Erickson A, Propst M, North A, Sherman CD, Mufti NA, Reed WF, Corash L. Stored red blood cell viability is maintained after treatment with a second-generation S-303 pathogen inactivation process. *Transfusion*. 2011; 51:2367–76. [PubMed: 21569044]
9. Cancelas JA, Gottschall JL, Rugg N, Graminske S, Schott MA, North A, Huang N, Mufti N, Erickson A, Rico S, Corash L. Red blood cell concentrates treated with the amustaline (S-303) pathogen reduction system and stored for 35 days retain post-transfusion viability: results of a two-centre study. *Vox Sang*. 2017; 112:210–8. [PubMed: 28220519]
10. Cancelas JA, Rugg N, Fletcher D, Pratt PG, Worsham DN, Dunn SK, Marschner S, Reddy HL, Goodrich RP. *In vivo* viability of stored red blood cells derived from riboflavin plus ultraviolet light-treated whole blood. *Transfusion*. 2011; 51:1460–8. [PubMed: 21275998]

11. Mock DM, Lankford GL, Widness JA, Burmeister LF, Kahn D, Strauss RG. Measurement of red cell survival using biotin-labeled red cells: validation against ⁵¹Cr-labeled red cells. *Transfusion*. 1999; 39:156–62. [PubMed: 10037125]
12. An G, Widness JA, Mock DM, Veng-Pedersen P. A novel physiology-based mathematical model to estimate red blood cell lifespan in different human age groups. *AAPS J*. 2016; 18:1182–91. [PubMed: 27215601]
13. Dziegiel MH, Koldkjaer O, Berkowicz A. Massive antenatal fetomaternal hemorrhage: evidence for long-term survival of fetal red blood cells. *Transfusion*. 2005; 45:539–44. [PubMed: 15819674]
14. Sebring ES, Polesky HF. Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects. *Transfusion*. 1990; 30:344–57. [PubMed: 2190367]
15. Mock DM, Widness JA, Veng-Pedersen P, Strauss RG, Cancelas JA, Cohen RM, Lindsell CJ, Franco RS. Measurement of posttransfusion red cell survival with the biotin label. *Transfus Med Rev*. 2014; 28:114–25. [PubMed: 24969019]
16. Recommended method for radioisotope red-cell survival studies. International Committee for Standardization in Haematology. *Br J Haematol*. 1980; 45:659–66. [PubMed: 7426443]
17. Mock DM, Lankford GL, Matthews NI, Burmeister LF, Kahn D, Widness JA, Strauss RG. Accelerated removal of antibody-coated red blood cells from the circulation is accurately tracked by a biotin label. *Transfusion*. 2012; 52:1097–105. [PubMed: 22023312]
18. Widness JA, Nalbant D, Matthews NI, Strauss RG, Schmidt RL, Cress GA, Zimmerman MB, Mock DM. Tracking donor RBC survival in premature infants: agreement of multiple populations of biotin-labeled RBCs with Kidd antigen-mismatched RBCs. *Pediatr Res*. 2013; 74:689–97. [PubMed: 24108188]
19. Mock DM, Matthews NI, Zhu S, Strauss RG, Schmidt RL, Nalbant D, Cress GA, Widness JA. Red blood cell (RBC) survival determined in humans using RBCs labeled at multiple biotin densities. *Transfusion*. 2011; 51:1047–57. [PubMed: 21062290]
20. Mock DM, Matthews NI, Zhu S, Burmeister LF, Zimmerman MB, Strauss RG, Schmidt RL, Nalbant D, Freise KJ, Veng-Pedersen P, Widness JA. Red blood cell (RBC) volume can be independently determined *in vivo* in the sheep using ovine RBCs labeled at different densities of biotin. *Transfusion*. 2010; 50:2553–64. [PubMed: 20561297]
21. Mock DM, Matthews NI, Zhu S, Burmeister LF, Zimmerman MB, Strauss RG, Schmidt RL, Nalbant D, Cress GA, Widness JA. Red blood cell (RBC) volume can be independently determined *in vivo* in humans using RBCs labeled at different densities of biotin. *Transfusion*. 2011; 51:148–57. [PubMed: 20630041]
22. Moroff G, Sohmer PR, Button LN. Proposed standardization of methods for determining the 24-hour survival of stored red cells. *Transfusion*. 1984; 24:109–14. [PubMed: 6710582]
23. Klein, HG., Anstee, DJ. Mollison's blood transfusion in clinical medicine. Klein, HG. Mollison, PL. Anstee, DJ., Mollison, PL., editors. Malden, MA: Blackwell Pub; 2005. p. 352-405.
24. Arbuckle TE, Wilkins R, Sherman GJ. Birth weight percentiles by gestational age in Canada. *Obstet Gynecol*. 1993; 81:39–48. [PubMed: 8416459]
25. Floss AM, Strauss RG, Goeken N, Knox L. Multiple transfusions fail to provoke antibodies against blood cell antigens in human infants. *Transfusion*. 1986; 26:419–22. [PubMed: 3765033]
26. Roseff, SD., editor. American Association of Blood Banks. Pediatric Transfusion: A Physician's Handbook. Bethesda, MD: AABB; 2006. p. 1-52.
27. Glynn SA. The red blood cell storage lesion: a method to the madness. *Transfusion*. 2010; 50:1164–9. [PubMed: 20598098]
28. Burger P, Hilarius-Stokman P, de Korte D, van den Berg TK, van Bruggen R. CD47 functions as a molecular switch for erythrocyte phagocytosis. *Blood*. 2012; 119:5512–21. [PubMed: 22427202]
29. Lauber K, Blumenthal SG, Waibel M, Wesselborg S. Clearance of apoptotic cells: getting rid of the corpses. *Mol Cell*. 2004; 14:277–87. [PubMed: 15125832]
30. Moroff G, Holme S, AuBuchon JP, Heaton WA, Sweeney JD, Friedman LI. Viability and in vitro properties of AS-1 red cells after gamma irradiation. *Transfusion*. 1999; 39:128–34. [PubMed: 10037121]

Figure 1.

24-hr post-transfusion recovery decreases with duration of storage for adults (n=86) but not for infants (n=42). Slope of the linear regression for healthy adults (black solid line) significantly differed from zero ($P<0.0001$) and from that of the very low birth weight infants ($P=0.04$). Data for all three tiers of allogeneic BioRBC densities were used for the infant regression adults received autologous ^{51}Cr -labeled BioRBCs): Low BioRBC density (gray dotted line); Medium BioRBC density (gray solid line); and High BioRBC density (black dashed line).