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Transfusion of older stored blood and risk of death: a meta-analysis

Dong Wang^{1,3}, Junfeng Sun¹, Steven B. Solomon¹, Harvey G. Klein², and Charles Natanson¹

¹Critical Care Medicine Department, National Institutes of Health, Bethesda, MD, USA

²Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD, USA

³Anesthesia and Critical Care Medicine Department, West China Hospital of Sichuan University, Cheng Du, China

Abstract

BACKGROUND—Blood for transfusion is stored for up to 42 days. Older blood develops lesions and accumulates potentially injurious substances. Some studies report increasing toxicity as blood ages. We assessed the safety of transfused older versus newer stored blood.

STUDY DESIGN AND METHODS—PubMed, Scopus and Embase were searched using terms *new* and *old* and *red blood cell* and *storage* through May 6, 2011 for observational and randomized controlled studies comparing outcomes using transfused blood having longer and shorter storage times. Death was the outcome of interest.

RESULTS—Twenty-one studies were identified, predominantly in cardiac surgery (n=6) and trauma (n=6) patients, including 409,966 patients. A test for heterogeneity of these studies' results was not significant for mortality ($I^2=3.7\%$, $p=0.41$). Older blood was associated with a significantly increased risk of death [odds ratio (OR) 1.16; 95% confidence interval (CI) (1.07, 1.24)]. Using available mortality data, 97 (63, 199; 95% CI) patients need to be treated with only new blood to save one life. Subgroup analysis of these trials indicated the increased risk was not restricted to a particular type of patient, size of trial, or amount of blood transfused.

CONCLUSION—Based on available data, use of older stored blood is associated with a significantly increased risk of death.

INTRODUCTION

In 1818, the British obstetrician James Blundell performed the first successful blood transfusion using small volumes of freshly drawn blood.¹ Since that time, transfusion of red blood cells (RBCs) has become one of the most important therapies in clinical medicine. The World Health Organization reported that 80.7 million units of blood were collected in 167 countries surveyed between 2004 and 2005.² Approximately 15 million units of whole blood are collected in the United States each year.³ In order to maintain adequate inventories

Correspondence and reprint requests: Dong Wang Critical Care Medicine Department Clinical Center, National Institutes of Health 9000 Rockville Pike Building 10, Room 2C145 Bethesda, MD 20892 Telephone: (301) 496-9320 Fax: (301) 480-5493 wangd4@cc.nih.gov..

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of RBCs of the appropriate type where it is needed, blood must now be processed, shipped, and stored at refrigerated temperature.

For the past 50 years, the primary aim of blood storage research has been to extend the RBC shelf life in order to maximize availability of a relatively scarce and perishable product. Modified storage solutions, containers, and processing procedures have doubled the shelf life to the current six weeks.⁴⁻⁶ This effort has proved successful. In the last US national survey, less than five percent (and less than one quarter of one percent of group O) of RBCs outdated, and fewer than five percent of hospitals surveyed reported canceling elective surgery for even one day because compatible blood was unavailable.⁷

Less attention has been paid to maintaining and assessing the physiological efficacy of refrigerated RBCs. For licensure in the US, a variety of in vitro assays to evaluate RBC structure and composition has become customary, but the Food and Drug Administration's (FDA's) "gold standard" requires that at least 75% of the cells remain in the circulation 24h after infusion, and that hemolysis not exceed 1% at the end of the approved storage period.⁸ Though 6-week-old blood meets the current FDA criteria, this "old" blood may not be as safe and effective as blood stored for shorter durations. Refrigerated storage results in a "storage lesion" that is reflected by metabolic derangements, RBC shape change, rheologic changes, loss of membrane carbohydrates, oxidative injury to lipids and proteins, changes in oxygen affinity and delivery, increased adhesion of RBCs to endothelial cells, and reduced RBC lifespan as well as the secondary risks of accumulating concentrations of potassium and plasticizer, and shedding of active proteins, lipids, and microvesicles.⁹ These changes that take place with the aging of stored blood raised concern that stored older RBCs could increase patient risks.

At present, most adult and pediatric patients requiring transfusion receive blood of their specific type with the oldest compatible unit available given first.¹⁰ This first-in, first-out principle was designed to conserve a limited and perishable blood supply. If RBC's that have been stored for an extended period were found to pose clinically significant risks, the current strategies for blood storage and transfusion used worldwide would come into question. Reducing the maximum shelf life for RBCs would involve new approaches to inventory management and result in major operational and financial consequences.¹¹

Published retrospective and prospective studies have raised concern that patients receiving older blood have an increased mortality risk compared to patients receiving newer stored units. Meta-analyses have struggled to summarize these studies because of variability in methods of reporting data in individual studies. In an attempt to summarize and give a full description of the previous published transfusion experience, we have used a methodology that allows the study endpoints to be comparable. Our primary objective is to determine the effect of storage on mortality and our secondary objective is to determine if effects are restricted to any subgroups.

MATERIALS AND METHODS

Data Sources, Searches and Study Selection

Three databases (PubMed, Scopus and Embase) were searched (all 3 were last searched May 6, 2011) to find all relevant articles published comparing patients receiving transfusions with new and old stored blood. The search was limited to "Humans" and publications in "English" but not to "Date" with additional search terms detailed for each database (see Appendix Table 1, Online Supplement), resulting in a total of 2640 articles. After removing duplicate articles, 1348 unique article citations remained. Each of these articles was reviewed. Trials were included if they compared survival rates after being transfused with

blood that was stored over different durations in days, one having longer storage times (“old blood”) and the other having a shorter storage time (“new blood”). To perform a comprehensive analysis, studies were only excluded if they did not have mortality data, or did not refer to the age of the stored blood transfused, or the study was subsequently retracted. A total of 21 trials met these inclusion criteria (Fig 1).

Data Extraction

Two of us independently (DW and JS) reviewed the included studies. A third author (CN) resolved any discrepancies. Mortality was selected as the primary endpoint of interest. We also abstracted other descriptive data from included trials (Table 1) such as year published, enrollment dates, study design, population studied, volume of blood transfused, age of blood stored and solution used, if blood was leukoreduced, mortality endpoints used, and if there were severe adverse events.

Data Synthesis and Analysis

In order to compare the effects of old versus new stored blood transfusion on the odds ratio (OR) of death across studies, each individual study was summarized as follows:

When both count data (survivor or non-survivor) and adjusted results [hazard ratio (HR), odds ratio (OR), or relative risk (RR)] were provided, the count data were used to calculate OR (old vs. new stored blood) for the analysis.¹²⁻²⁰ Adjusted HR^{12, 18, 20-22} and RR¹³ were converted to OR (proportional hazard was assumed for HR). Using the adjusted ORs instead of counts in sensitivity analyses, we obtained similar results.

If more than one way of dividing the subgroups were reported, the division that provided the greatest degree of age separation between old and new stored blood was used.^{14,15,18,19,21-24} When count data were used and there were more than 2 blood age groups, only the patients from the newest and oldest groups were used in this meta-analysis.^{17-19,23, 24} When the estimated effect (e.g., OR) used was derived from multivariate analysis, all patients from that study were used in this meta-analysis.

When more than one cut-off value was used to divide 2 groups of stored blood, the cut-off closest to 21 days was used.^{17,20}

If more than one way of calculating blood age was used, the result, based on average blood age was used.²⁵

When each transfused group (newest or oldest) was compared to a third group (an intermediate blood age group or “no transfusion” group), the comparison of oldest versus newest was calculated (based on the estimates and standard errors from the newest (or oldest) versus no transfusion) using an approximate t test.^{22,26}

When the estimated effect was based on a continuous blood age (e.g., OR of death when blood age increases by 1 day), the estimated effect size of third-quartile blood age versus first-quartile blood age was used in the analysis.²⁵ For one study,²⁷ the continuous blood age used was the sum of “proportionate age” (i.e., age divided by 42 days) of each blood unit. In this case, the first and third quartiles of this calculated blood age were used to calculate the estimated effect size. Adverse events were defined a priori and in studies meeting inclusion criteria were reported if significant and combined if appropriate when reported in at least two studies.

Heterogeneity among studies was assessed using the Q statistic and I² value.²⁸ A random effects model was used and not the fixed-effects model to estimate the overall effect of old

stored blood versus new stored blood transfusion because we assumed it was unlikely effects would be identical across studies and wanted to make inferences for all potential studies.²⁹ Summary ORs are reported when heterogeneity was low ($< 30\%$ I^2 value). Subgroup analysis with different conditions was done using random-effects meta-analysis. Publication bias was assessed by funnel plot and Egger's regression^{30,31} (Fig 2). Conventional forest plots were prepared, with the sizes of point estimates proportional to the inverse variance of each estimate. The overall estimates for the increase in mortality with older transfused blood based on counts data versus adjusted results (among studies that provided both) were compared using exact Wilcoxon matched pairs signed rank test (StatXact-9, Cytel Software Corp., Cambridge, MA). All analyses were performed using Comprehensive Meta-analysis version 2 (<http://www.meta-analysis.com/>) except noted otherwise.

RESULTS

Study Characteristics

Twenty-one studies^{10,12-27,32-35} that were published from 2001 to 2011 met inclusion criteria (Fig. 1, Table 1). Three of these studies were randomized controlled trials³³⁻³⁵ and eighteen observational studies, six prospective studies,^{10,12,15,21,23,24} and twelve retrospective.^{13,14,16-20,22,25-27,32} The patients were enrolled in these studies from as early as 1991 to 1993 to as late as 2000-2009. The three randomized controlled trials³³⁻³⁵ were much smaller in size, ranging from 17 to 57 patients and the observational studies^{10,12-27,32} much larger, ranging in size from 66 to 387,130 patients. Most studies were in cardiac surgery^{14,16,18,20,25,34} ($n = 6$) or trauma patients^{13,17,19,26,27,35} ($n = 6$). The age of new blood in these 21 studies ranged from 1.6 days to 21 days and the old blood, 9 days to 31 to 42 days. The blood was reported to be leukoreduced in 16^{10,12-15,18-21,23,24,26,27,33-35} out of 21 studies. The average volume of blood transfused per patient in studies ranged from 1 unit to almost 10 units.

Effect of Old versus New Stored Blood on Mortality

There was a total of 555 deaths among 7611 patients transfused with new blood (7.3%) and a total of 774 deaths among 9114 patients transfused with old blood (8.5%) from sixteen studies that provided such data.^{10,12-20,23,24,32-35} There was no significant heterogeneity among these 21 studies for the mortality endpoint ($I^2 = 3.7\%$; $p = 0.41$) (Fig 3). The overall estimate of mortality with transfusion of old blood compared to new stored blood was a significantly increased odds ratio (OR) of death, 95% confidence interval (CI) of 1.16 (1.07, 1.24). Using the available mortality data from the current studies (7.3% for new blood group), based on our meta-analysis, the number needed to treat (NNT) (95% CI) is 97 (63, 199) patients with only new blood to save one life.

Effect of Old versus New Stored Blood on Severe Adverse Effects

Seven^{10,16,17,24-26,33} of 21 studies included in our meta-analysis described one or more severe adverse effects: intubated ventilation > 72 hours, pneumonia, renal failure, sepsis, acute respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome (MODS), and deep vein thrombosis. Averaging over the three studies reporting the incidence of MODS^{10,16,24} and the three studies reporting the incidence of pneumonia,^{16,25,26} there was no significant heterogeneity among these studies ($I^2 = 0\%$; $p = 0.44$ and $I^2 = 0\%$; $p = 0.50$, respectively) and the overall estimate showed a significant increase in MODS and pneumonia with transfusion of old stored blood OR 2.26, 95% CI (1.56, 3.25) and 1.17 (1.08, 1.27), respectively. Two studies found a non-significant overall increase in the incidence of ARDS^{26,33} with transfusion of older stored blood (Fig. 4). Three studies reported the incidence of renal dysfunction; two^{16,26} found significant increases with transfusion of older blood, and the third,²⁵ no significant effect of stored blood age

transfused. Two studies reported the incidence of sepsis; one found a significant increase with transfusion of older stored blood,¹⁶ the other no significant effect of the age of stored blood transfused.³³ The above studies of either renal dysfunction or sepsis could not be combined because of significant heterogeneity among studies for each condition ($I^2 = 62.6\%$ and 77.6% respectively). The single studies reporting intubated ventilation for > 72 hours¹⁶ and deep vein thrombosis¹⁷ both found a significant increase with transfusion of older stored blood.

Subgroup Analysis

Figure 5 shows the odds ratio of death according to different patient categories. In trauma patients, cardiac surgery patients and the non-cardiac, non-trauma patients (including those with anemia, cardiac disease, undergoing colorectal surgery, ICU patients, premature infants, and for one study, all patients regardless of type of illness that were transfused at least one unit), the OR of death significantly increased in all three subgroups with old vs. new blood. There was no significant heterogeneity among studies in any of the three subgroups or significant difference comparing these subgroup' increases in the OR of death ($p = 0.60$). In cardiac surgery patients^{14,16,18,20,25,34} the OR (95% CI) of death with old verse new blood was 1.26 (1.04, 1.53), somewhat larger than the overall estimate for the 21 studies. In trauma patients^{13,17,19,26,27,35} the OR (95% CI) of 1.18 (1.02, 1.35) was very similar to the overall estimate of the increase in mortality with old vs. new blood. The smallest increase in OR of death with old vs. new blood was in patients with non-trauma, non-cardiac surgery^{10,12,15,21-24,32,33} related transfusions with OR (95% CI) of 1.12 (1.00, 1.26).

Figure 6 shows odds ratio of death for pediatric and adult patients. There was no significant heterogeneity among the studies in either of these two subgroups ($I^2 = 0\%$; $p = 0.86$ and $I^2 = 14.9\%$; $p = 0.28$, respectively). In the pediatric group,^{10,24,33} the overall estimate of death was increased but not significantly, OR (95% CI) 1.43 (0.76, 2.69) and in the adult group,^{12-23,25-27,32,34,35} the overall estimate of mortality was significantly increased with transfusion of older blood 1.16 (1.07, 1.25). There was no significant difference in overall estimates comparing adults and children ($p = 0.51$); however, the estimate was nominally larger in children and less significant potentially because of a lack of power, there being far fewer pediatric studies.

Figure 7 shows the odds ratio of death according to studies that provided counts data^{10,12-18,20,23,24,26,32-35} (survivor/non-survivor) or adjusted mortality results.^{19,21,22,25,27} There was no significant heterogeneity ($I^2 = 12.6\%$, $p = 0.31$ and $I^2 = 0\%$, $p = 0.57$, respectively) within either of these two subgroups and no significant difference in the OR of death (comparing the two subgroups) ($p = 0.55$). In the counts group the OR (95% CI) was 1.20 (1.05, 1.36); in the adjusted group, 1.14 (1.04, 1.26). There were eight studies^{12-15,17-20} which provided both counts data and adjusted mortality analysis comparing new and old stored blood transfusion. The overall estimates for the increase in mortality with older transfused blood were similar ($P = 0.30$) comparing counts, 1.14 (0.96, 1.36), $p = 0.13$ and adjusted mortality analysis 1.42 (1.00, 2.01), $p = 0.05$. If there is any difference at all, adjustment resulted in a larger treatment effect that was more significant than counts. However there was more marked heterogeneity using adjusted mortality compared to counts (adjusted $I^2 = 66.6\%$, $p = 0.004$ versus counts $I^2 = 23.4\%$, $p = 0.24$), potentially reflecting the different methods across studies used to adjust mortality. Thus counts give a similar but somewhat more conservative estimate of the overall effect of older transfused stored blood on mortality with less variability.

From 2001 to 2008, there were 11 studies published comparing the effect of age of stored blood on mortality^{12,16,18,19,23,25-27,33-35} approximately one per year. From 2009 to 2011,

there were 10 such studies published^{10,13-15,17,20-22,24,32} approximately two per year. An observational study of 6002 cardiac surgery patients from a single institution published in 2008 showed a highly significant increase in mortality with older stored transfused blood, received wide publicity in the medical and lay press, and may have influenced this doubling.¹⁶ However, the increase in mortality with older stored transfused blood OR (95% CI) was similar ($p = 0.81$) in the studies published before 2009 [1.18 (1.06, 1.31); $p = 0.03$ ($I^2 = 23.8\%$; $p = 0.22$)] and in those published 2009 to 2011 [1.15 (1.01, 1.31); $p = 0.03$ ($I^2 = 0\%$; $p = 0.50$)].

We further divided these 21 studies into nine large studies ($n > 500$)^{13,14,16,18-22,25} and twelve small studies ($n \leq 500$).^{10,12,15,17,23,24,26,27,32-35} There was no significant heterogeneity in each subgroup ($I^2 = 0\%$, $p = 0.49$ and $I^2 = 17.4\%$, $p = 0.27$, respectively). The OR (95% CI) were similar for large and small studies ($p = 0.92$), being 1.17 (1.06, 1.30) and 1.16 (1.01, 1.34), respectively. We also divided these 21 studies according to the number of red blood cell units that were transfused to patients on average in each study: in ten trials ≤ 3 units group,^{12,14-16,21,22,24,25,27,34} in seven trials > 3 units group,^{10,13,17-19,26,35} and in four trials^{20,23,32,33} this information was not provided. There was significant heterogeneity among the trials receiving ≤ 3 units ($I^2 = 49.0\%$, $p = 0.04$), but not among the studies receiving > 3 units, and unknown number of units ($I^2 = 0\%$, $p = 0.65$ and $I^2 = 0\%$, $p = 0.85$, respectively). The OR (95% CI) were very similar ($p = 0.96$), regardless of number of units transfused, 1.19 (1.05, 1.35), 1.16 (1.00, 1.35) and 1.20 (0.87, 1.66), respectively. In five studies^{15,18,19,23,24} our a priori analytic plan reduced the sample size; if we include all these patients in our analysis, the OR (95% CI) for mortality is 1.20 (1.10, 1.31), very similar to our overall estimate 1.16 (1.07, 1.24) (Fig. 3). Finally, with the removal of the three small randomized controlled trials,³³⁻³⁵ the OR (95% CI) for the remaining 18 observational studies of 1.16 (1.08, 1.24) compared to the overall estimate (Fig. 3) is essentially unchanged.

Publication Bias

In Figure 2 is shown a funnel plot. The 21 studies in our meta-analysis are equally distributed in Figure 2 on both sides of the line, representing the significant increase in OR of death with old vs. new blood. Moreover, the Eggers test for publication bias is non-significant. This indicates absence of statistical evidence for a publication bias (i.e. that unpublished studies exist that are influencing the finding of an increase in mortality with old vs. new blood).

DISCUSSION

Our literature search and inclusion/exclusion criteria resulted in a total of 21 unique studies^{10,12-27,32-35} that compared survival rates with blood that was stored over different durations in days, one being longer “old” and one being shorter “new” blood. These studies were published from 2001 to 2010, included 18 observational studies,^{10,12-27,32} 6 prospective^{10,12,15,21,23,24} and 12 retrospective,^{13,14,16-20,22,25-27,32} and 3 randomized controlled trials³³⁻³⁵. The studies enrolled patients from 1991 to 2009 and ranged in size from 17 to 387,130 patients. Sixty percent of the studies were in cardiac surgery and trauma patients, but in the remaining forty percent of studies the patients varied from neonates to patients with anemia or colorectal cancer (Table 1). Nonetheless, the findings across these 21 studies were statistically very similar ($I^2 = 3.7\%$, $p = 0.41$) (Fig. 3) and showed a significant increase in mortality for those receiving old blood rather than new blood [odds ratio 95% CI 1.16 (1.07, 1.24)]. Taking into account both the mortality data found in these studies and the amount of blood transfusions per patient (range from 1-10 units on average), our data suggest one would need to treat 97 patients [95% CI, (63, 199)] exclusively with newly stored blood to save one life.

We performed multiple analyses to determine whether our results were consistent across potentially important subgroups. In our meta-analysis, approximately thirty percent of the studies were in trauma patients,^{13,17,19,26,27,35} thirty percent in cardiac surgery patients,^{14,16,18,20,25,34} and forty percent were a mix of varied populations.^{10,12,15,21-24,32,33} The results were similar comparing these three subgroups (Fig. 5) and very consistent with the overall increase in mortality found with old blood vs. new blood (Fig. 3). In our meta-analysis, the results of three pediatric patient studies,^{10,24,33} compared to the 18 adult studies,^{12-23,25-27,32,34,35} were similar (Fig. 6) and consistent with the overall increase in mortality found with transfusion of older stored blood (Fig. 3). In studies having 500 or > 500 patients, as well as in studies of patients receiving on average 3 units versus > 3 units per patient, the results in all these subgroups were likewise similar to the overall findings of our study showing old blood versus new blood increases mortality. In studies published before 2009 (n = 11)^{12,16,18,19,23,25-27,33-35} and afterward 2009 to 2011 (n = 10),^{10,13-15,17,20-22,24,32} the increases in mortality with older stored blood were statistically significant and similar to the overall findings in this meta-analysis. Many of these studies were observational; however, we found that if we examined only the five studies^{19,21,22,25,27} using adjusted mortality rates, the results were similar (p = 0.55) to the 16 studies^{10,12-18,20,23,24,26,32-35} that used count data (survivor/non-survivor) [1.14 (1.04, 1.26) vs. 1.20 (1.05, 1.36)] (Fig. 7), and were consistent with the overall findings of our study that older blood increases mortality (Fig. 3). Moreover, if we compare the studies that reported both counts and adjusted mortality (Fig. 7 top), the results are similar and comparable to the overall finding showing old blood versus new increases mortality (Fig. 3). Finally, if we remove the three small-randomized controlled trials, the overall results are unchanged. Thus, our results are statistically very similar across individual trials, similar with different types of analysis, and also very consistent across multiple different clinically important subgroups.

Only 7^{10,16,17,24-26,33} of the 21 studies in our meta-analysis reported serious adverse events (Fig. 4). Three studies^{16,25,26} suggested an overall significant increase in pneumonia and three other studies^{10,16,24} showed an overall increase in MODS associated with transfusion of old blood. Two^{16,26} of three^{16,25,26} studies showed significant increase in renal failure and one¹⁶ of two^{16,33} studies, a significant increase in sepsis with transfusion of older blood. The available data on serious side effects suggest, similar to mortality, an increase in risk using old versus new blood.

Four previously published reviews³⁶⁻³⁹ examined the available clinical studies comparing the effects on outcome of old versus new blood. These four papers were accepted for publication from September 2008 to August 2009. Since that time, nine studies,^{10,13-15,20-22,24,32} including 400,042 patients providing mortality data comparing old versus new blood have been published. These nine studies^{10,13-15,21,22,24,33} are included in our main analysis but not the four previous reviews.³⁶⁻³⁹ Three of these four previous reviews did not include a formal meta-analysis³⁶⁻³⁸. All three indicated that the currently available data set was insufficient to draw the conclusion that old blood is harmful. It is not possible to compare these studies and ours, since the data sets are markedly different and no formal analysis was done in these three other studies. One of these three reviews³⁸ stated the studies examined were too heterogeneous to allow an overall analysis. No formal analysis is provided to support this conclusion..

One review³⁹ did a formal meta-analysis but only analyzed subgroups of studies that were homogenous. This study concluded that the available data are not adequate to confirm the suspicion that older blood increases morbidity and mortality.³⁹ We believe that overall our results do not differ from this meta-analysis, but rather represent an increase in numbers and therefore in power. More studies are included in our meta-analysis and each of our subgroup analyses. For example, in this previous review,³⁹ an OR (95%) CI for mortality was reported

for trauma patients of 1.24 (1.06 – 1.44) based on three studies markedly similar to our result 1.18 (1.02 – 1.35) based on six studies which reached statistical significance (Fig.5).

Most of the available data in our meta-analysis derive from observational studies and conclusions must be interpreted cautiously because of the potential for unintentional bias. It is possible, for example, that some practitioners selected fresher units for children and older units for elderly patients with a poor prognosis, or there could be some other systematic unknown confounder across studies giving us this signal. However, the consistency of the data in this meta-analysis supports the hypothesis that older stored blood increases mortality. Nonetheless, until well designed, multicenter, adequately powered, randomized controlled trials confirm these findings, the evidence is inadequate to institute major changes in blood collection and transfusion practice. Three such trials, one in cardiac surgery patients, one in critically ill patients and another in neonates have been initiated.⁴⁰⁻⁴²

Extrapolation of these findings to the general population should be done with caution. Many of these studies involved critically ill trauma victims or patients with serious cardiac disease. Older blood may amplify the injury of certain diseases or interventions. It is possible that old blood acts as a “second hit” for patients with severely compromised organ function or immune competence.⁴³ If the risk of death from a unit of blood as estimated by the US Government Accountability Office is of the order of magnitude of one in 10,000, and transfusion is the only cause of death for a general patient population, the mortality for that population would be 0.01%.⁴⁴ Using this estimate of mortality and the OR estimate we found in our meta-analysis between old and new blood, one would have to treat 69,428 patients with exclusively new blood to save one life. If old blood increases risk, then these two estimates of 97 versus 69,428 likely encompass the number of patients needed to be treated with exclusively newer blood to save one life. The ongoing randomized controlled trials should help determine which one of the above estimates is closer to the actual risk for the “average” patient in need of a transfusion and for specific categories of patients whose clinical status might render them unusually vulnerable to changes that occur during the storage of RBCs.

Clinical studies comparing day-old blood with blood at the end of its storage life are operationally difficult and ethically challenging. RBCs must be processed, tested and shipped prior to issue for transfusion which places some limit on the extent of “freshness.” Blood stored for 42 days deteriorates by most measures of red blood cell function and older stored blood has been associated with increased mortality in several published studies. It is hard to imagine that a patient given this information would consent to receive only blood at the very end of its shelf life. The ongoing human clinical trials have been designed appropriately to compare fully tested, compatible newer blood with current transfusion practice or with older RBCs of mixed age available in the hospital inventory.^{40,41,42} Animal models might be better able to assess the clinical differences, if any, between the very youngest units and those at the end of shelf life and if older blood is found to increase risks, help define after how many days of storage and in which type of clinical circumstances.

Two endpoints other than morbidity and mortality have been investigated in relation to the age of stored blood transfused: tumor recurrence and oxygen delivery. Edna and Bjerkeset⁴⁵ found that blood storage time had no effect on local recurrences and distant metastases in 336 patients who had colorectal cancer surgery, yet Mynster and Nielsen¹² found that, compared with patients who did not undergo transfusion, those who received allogeneic blood stored for less than 21 days had increased cancer recurrence after colorectal surgery; those who underwent transfusion with blood with a storage age older than 21 days had a recurrence rate similar to that of non-transfused patients. The clinical importance of the RBC storage lesion on oxygen delivery in adults varied, some studies suggesting the age of

blood has little effect on oxygen delivery, using measures as varied as neurocognitive function and gastric tonometry.⁴⁶⁻⁴⁸ Still other studies suggest impairment of oxygen delivery using measures such as cerebral oxygenation in patients with traumatic brain injury or peripheral tissue oxygenation in pediatric or elderly patients.^{23,49,50} Conclusions drawn using these two endpoints are much less consistent than what we found for mortality in association with older blood.

We conclude that the published clinical experience to date suggests that newer blood if used exclusively might save lives. This effect is based largely on observational studies and could be confounded by some unrecognized bias that should be addressed in the three randomized controlled trials ongoing testing older versus newer blood.⁴⁰⁻⁴² Nonetheless, the data available to date cause concern that our current blood banking storage practices may not adequately protect patients. If older RBCs are found to pose clinically significant risks, current systems of blood collection, storage and transfusion would need to be revised and would likely entail major operational and financial impacts. Blood collectors should be developing strategies to assure that blood availability can be maintained should our results be confirmed by the ongoing clinical trials.

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Appendix

Appendix Table 1

Online supplement search strategies used for each database

Data Base	Search Strategy	Terms	Records Found
PubMed	blood preservation[mesh] AND erythrocyte transfusion[mesh] AND time factors[mesh] Limits: Humans, English	MeSH terms (PubMed controlled vocabulary)	116
PubMed	("red cell"[ti] OR "red cells"[ti] OR "red blood cell"[ti] OR "red blood cells"[ti] OR "transfused blood"[ti] OR "transfused cells"[ti]) AND (duration[ti] OR storage[ti] OR stored[ti] OR age[ti] OR time[ti] OR old[ti] OR older[ti]) Limits: Humans, English		765
PubMed	("red cell"[ti] OR "red cells"[ti] OR "red blood cell"[ti] OR "red blood cells"[ti] OR "transfused blood"[ti] OR "transfused cells"[ti]) AND (duration[ti] OR storage[ti] OR stored[ti] OR age[ti] OR time[ti] OR old[ti] OR older[ti]) NOT medline[subset] Limits: English		42
Scopus	(TITLE("red cell" OR "red cells" OR "red blood cell" OR "red blood cells" OR "transfused blood" OR "transfused cells") AND TITLE(duration OR storage OR stored OR age OR time OR old OR older)) AND (LIMIT-TO(EXACTKEYWORD, "Human")) AND (LIMIT-TO(LANGUAGE, "English"))		813
EMBASE	'erythrocyte preservation'/exp/mj OR 'blood storage'/exp/mj AND 'blood transfusion'/exp/mj OR 'erythrocyte transfusion'/exp/mj AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim)	EMTREE terms (EMBASE controlled vocabulary)	328
EMBASE	'red cell':ti OR 'red cells':ti OR 'red blood cell':ti OR 'red blood cells':ti OR 'transfused blood':ti OR 'transfused cells':ti AND (duration:ti OR storage:ti OR stored:ti OR age:ti OR time:ti OR old:ti OR older:ti) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim)		576

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Figure 1.
Study selection

Figure 2. Funnel plot of studies included in meta-analysis

Each open circle represents one of 21 studies in our meta-analysis. The precision for each study is plotted versus log (OR). The circles are distributed equally around the solid vertical line with a solid diamond at the bottom, representing the overall treatment effect in this study. There is no skewed distribution of studies giving evidence for a publication bias. Eggers test for publication bias (or small study effect) is non-significant ($P=0.43$) and confirm what is seen on visual expectedness.

Figure 3. Mortality

The size of the data markers is proportional to the inverse variance of each point estimate.

Abbreviations: ARDS, acute respiratory distress syndrome; MODS, multiple organ dysfunction syndrome.

Figure 4.
Severe adverse effects.

Figure 5.
Subgroup analysis (Patient types).

Figure 6.
Subgroup analysis (Pediatric and Adult).

Figure 7.
Subgroup analysis (Adjusted mortality versus counts).

Characteristics of studies included in meta-analysis

Table 1

Author (Reference)	Year Published	Years of Enrollment	Study Type	Study Population	No. of Patients in study	No. of Patients in Metaanalysis *	Average Transfusion Volume per Patient (units of PRBC)	Age of Stored Blood Compared [†]		Blood Storage Solution [‡]	Leuko-reduced	Mortality
								New Blood (days)	Old Blood (days)			
Van Straten ¹⁴	2011	1998–2007	OBR	Cardiac surgery	3597	3141	new=2.6, old=2.4	< 14	14	SAGM	Yes	30-d mortality
Pettit ¹⁵	2011	2008	OBP	ICU patient	757	379	2	11	28	NR	Partially	Hospital mortality
Edgren ²²	2010	1995–2002	OBR	Transfused at least one RBC unit	387,130	387,130	2	< 9	> 30	SAGM	NR	Short term mortality (1 week)
Eikelboom ²¹	2010	2002–2006	OBP	Cardiac disease	4933	4933	3	< 10	31–42	AS–3	Yes	Hospital mortality
Robinson ²⁰	2010	1999–2005	OBR	Cardiac surgery	909	712	NR	21	> 21	NR	Yes	30-d mortality
Weinberg ¹³	2010	2000–2009	OBR	Trauma	1647	1647	new=2.9, old=3.4	< 14	14	NR	Yes	Hospital mortality
Karam ¹⁰	2010	2004–2005	OBP	PICU patient	296	296	new=2.6, old=5.5	< 14	14	NR	Partially	28-d mortality
Gauvain ²⁴	2010	2001–2005	OBP	PICU patient	455	224	new=1.2, old=2.3	7	> 21	NR	Yes	28-d mortality
Van Buskirk ³²	2009	NR	OBR	ICU patient	298	298	NR	< 8	> 14	NR	NR	Hospital mortality
Spinella ¹⁷	2009	2004–2007	OBR	Trauma	202	176	9	<21	21	NR	NR	Hospital mortality
Koch ¹⁶	2008	1998–2006	OBR	Cardiac surgery	6002	6002	2	14	> 14	NR	NR	Hospital mortality
Weinberg ²⁶	2008	2000–2007	OBR	Trauma	430	430	5.2	< 14	14	NR	Yes	Hospital mortality
Yap ²⁵	2008	2001–2007	OBR	Cardiac surgery	670	670	3	8	19	NR	NR	Hospital mortality
Weinberg ¹⁹	2008	2000–2007	OBR	Trauma	1813	1169	4.95	<14	14	NR	Yes	Hospital mortality
Leal-Naval ²³	2008	2004–2006	OBP	Anemia	66	34	NR	< 10	> 19	SAGM	Yes	ICU mortality
Van-De-Watering ¹⁸	2006	1993–1999	OBR	Cardiac surgery	2732	1895	4.77	< 18	18	SAGM	Yes	30-d mortality
Murrell ²⁷	2005	2001–2002	OBR	Trauma	275	275	3	NR	NR	NR	Yes	Hospital mortality
Fernandes-Da-Cunha ³³	2005	2002–2003	RCT	Prenature infant	52	52	NR	1. 6	9	CPDA–1	Yes	Hospital mortality
Hebert ³⁴	2005	1999–2001	RCT	Cardiac surgery	57	57	new=3, old=2	4	19	CPD–2, AS–3	Yes	Hospital mortality
Schulman ³⁵	2002	2000–2001	RCT	Trauma	17	17	new=9.3, old=10.6	<11	20	NR	Yes	Hospital mortality
Mynster ¹²	2001	1991–1993	OBP	Colorectal surgery	740	429	3	< 21	21	SAGM	Yes	Long term mortality

Abbreviations: NR, not reported; OBR, observational retrospective study; OBP, observational prospective study; RCT, randomized controlled trial; ICU, intensive care unit; PICU, pediatric intensive care unit; PRBC, packed red blood cells

* When multiple ages of stored blood were presented only the extremes were analyzed (see statistical method)

[†] See statistical method for how stored blood groups were determined for comparison

[‡] The numbers and letters indicate the type of storage solution: C=citrate, A=adenine, P=phosphate, D=dextrose, S=sodium chloride, M=mannitol, G=glucose