Transfusion of packed red blood cells at the end of shelf life is associated with increased risk of mortality – a pooled patient data analysis of 16 observational trials

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ABSTRACT

Observational studies address packed red blood cell effects at the end of shelf life and have larger sample sizes compared to randomized control trials. Meta-analyses combining data from observational studies have been complicated by differences in aggregate transfused packed red blood cell age and outcome reporting. This study abrogated these issues by taking a pooled patient data approach. Observational studies reporting packed red blood cell age and clinical outcomes were identified and patient-level data sets were sought from investigators. Odds ratios and 95% confidence intervals for binary outcomes were calculated for each study, with mean packed red blood cell age or maximum packed red blood cell age acting as independent variables. The relationship between mean packed red blood cell age and hospital length of stay for each paper was analyzed using zero-inflated Poisson regression. Random effects models combined paper-level effect estimates. Extremes analyses were completed by comparing patients transfused with mean packed red blood cell aged less than ten days to those transfused with mean packed red blood cell aged at least 30 days. sixteen datasets were available for pooled patient data analysis. Mean packed red blood cell age of at least 30 days was associated with an increased risk of in-hospital mortality compared to mean packed red blood cell of less than ten days (odds ratio: 3.25, 95% confidence interval: 1.27-8.29). Packed red blood cell age was not correlated to increased risks of nosocomial infection or prolonged length of hospital stay.

Introduction

Packed red blood cells (PRBCs) have a shelf life of 21-49 days depending on the additive solution used and jurisdiction.[1] During *in vitro* storage, PRBCs accumulate cellular and biochemical changes collectively called the red blood cell (RBC) storage lesion. These changes occur secondary to RBC metabolism under artificial conditions such as refrigeration temperatures, limited nutrient supply and absent clearance mechanisms. RBC storage lesions clearly disturb physiological mechanisms and lead to harm in *in vitro* tests and animal models.[2,3] Clinical data linking PRBC storage duration to adverse outcomes, such as mortality, nosocomial infection, prolonged hospital length of stay (HLOS), prolonged intensive care unit (ICU) length of stay, prolonged mechanical ventilation, acute renal failure and multiple organ dysfunction – have been consistently equivocal.[2,4] The majority of studies demonstrating the damage caused by prolonged PRBC storage have been retrospective and observational in nature.[5] Recent large randomized controlled trials (RCTs) have not linked PRBC storage duration to adverse outcomes.[6-9] However, the two types of studies tended to ask different questions— the former, "Is prolonged PRBC storage harmful?" *versus* the latter, "Are fresh PRBCs better than standard practice?". Furthermore, observational studies often had larger

Table 1. Data synthesis method.

Demographic variables	
Age	Calculated from date of birth and date of first transfusion
BMI	Calculated from height and weight
Ejection fraction	Classified as good >50%, fair 30-50%, poor <30%
Outcome variables	
Mortality	In-hospital mortality used whenever possible; when not reported, the mortality over the shortest duration used (e.g. 30-day mortality over 90-day mortality)
Surgical wound infection	General surgery: abdominal + perineal wound infections, intra-abdominal abscess Cardiothoracic surgery: sternal infection, anastomosis infection
Any infection	Any infectious outcome recorded including pneumonia, surgical wound infection, bloodstream infection, urinary tract infection
Duration of MV	Converted to days when reported as hours
HLOS	Calculated from hospital admission and discharge date
ILOS	Calculated from ICU admission and discharge date
MODS	Peak SOFA score ≥6 provided that SOFA score<6 prior to intensive care unit admission

BMI: body mass index; HLOS: hospital length of stay; ICU: intensive care medicine; ILOS: intensive care length of stay; MODS: multiple organ dysfunction syndrome; MV: mechanical ventilation; SOFA: sepsis-related organ failure assessment score.

sample sizes with older mean ages of transfused PRBC, making them uniquely positioned to identify small adverse effects owing to PRBC units at the end of shelf life.[10]

One of the key issues with combining data from observational studies is the diverse methods of describing aggregate PRBC age, ranging from mean PRBC age to dichotomization at "x" days to maximum PRBC age transfused.[5] Some paper level meta-analyses have used various adjustments with the aim of unifying aggregate PRBC age measurements and maximizing PRBC age differences between comparison groups.[11] However, the temporal effect of storage-induced adverse outcomes has been relatively unmapped, and there is no evidence that different measures of aggregate PRBC age are interchangeable in their association with clinical outcomes.[12] Moreover, the assumptions used to convert one aggregate measure to another (e.g., median to mean) could potentially lead to statistical inaccuracy. This issue could be abrogated *via* pooled patient data analysis allowing the use of one aggregate PRBC age measure. Pooled patient data analysis also touts improved subgroup analyses and consistency across studies compared to paper level analysis.[13]

This study analyzed pooled individual patient data (IPD) from 16 observational studies with the aim of quantifying the association of PRBC storage duration on mortality, nosocomial infection and HLOS. In representing 16 retrospective studies and over 17,000 patients – the study herein is one of the largest pooled patient data analysis completed for the investigation of storage-induced adverse PRBC transfusion outcomes to date.

Methods

Study selection

Institutional ethics approvals were sought from the University of Queensland and The Alfred Hospital prior to initiation of study. Observational studies reporting PRBC storage duration and clinical outcomes such as mortality, infection and HLOS were identified from PubMed and EMBASE using protocols described previously.[5] Corresponding investigators of each study were contacted to request the underlying patient-level dataset.

Data extraction and synthesis

Demographic, intervention and outcome variables reported in more than three studies were combined into one Microsoft Excel 2016 spreadsheet by author Monica S.Y. Ng. and checked by author Angela S.Y. Ng. Patients who did not receive any PRBC units were excluded from the dataset. Variables not reported in the desired format were calculated from primary data where available. Table 1 demonstrates adjustments made to synthesize the aggregate datasheet.[14] When individual PRBC unit ages were available, the aggregate ages of PRBC transfusions were expressed as mean age or maximum PRBC age. In so doing, aggregate PRBC ages were expressed in a time-independent manner for incorporation into logistic models.

Data analysis

A two-stage meta-analysis using IPD was used to account for differences between study cohorts in general analyses. This approach has been shown to increase statistical power and avoid ecological bias when compared with the traditional approach which pools study estimates.[15,16] In the first stage, the association between binary outcomes (such as in-hospital mortality or nosocomial infection) and PRBC age (expressed as mean PRBC age or maximum PRBC age) were calculated using binomial logistic regression for each study with age, sex and PRBC volume as continuous covariates. PRBC age (mean PRBC age, maximum PRBC age), recipient age and PRBC volume were incorporated as continuous variables, while sex was included as a binary variable. Each effect estimate was reported as an odds ratio (OR) with 95% confidence intervals (CI). Regarding HLOS, and due to an excessive number of zeroes, zero-inflated Poisson regression modelling was used to calculate the incidence rate ratio (IRR) for an additional day in hospital as a function of PRBC age (expressed as mean PRBC age or maximum PRBC age) for each paper. In the second stage, random effects models were used to combine the effect estimates for each paper. Funnel plots were generated for each analysis involving more than ten papers to assess for publication bias. This threshold was used as funnel

plots with ten or less studies have insufficient power to identify heterogeneity.[17] Sensitivity analyses were completed by calculating the adjusted OR for each geographical location (the Americas, Europe, other) and patient subgroup (cardiac surgery, intensive care unit (ICU), other). Time-lapse analyses involved adding studies sequentially to the random effects model in order of the recruitment period (i.e., from 1980-2011).

In the extremes analyses for in-hospital mortality and nosocomial infection, logistic regression was used to calculate an aggregate OR and 95% CI comparing patients transfused with mean PRBC aged less than ten days to those transfused with mean PRBC aged at least 30 days old. The ten day threshold for fresh PRBC was selected to align with the Red Cell Storage Duration Study (RECESS). The 30 day threshold for stored PRBC was selected to maintain relevance for jurisdictions which store PRBC for 35 (e.g., China, The Netherlands, UK) and 42 days (e.g., Australia, USA, Canada). Furthermore, *in vitro* research suggests that PRBC storage lesions become clinically significant up to day 28.[18] Subgroup analyses were completed to measure the modifying effects of leukoreduction status. Zero-inflated Poisson regression was used to calculate an aggregate IRR and 95% CI for HLOS extremes analysis. Age, sex and PRBC volume were included as covariates for each model. STATA™ (StataCorp 2017, version 15.0) was utilized for all analyses.

Results

Study characteristics

Using the search strategy as previously specified, 3285 abstracts were retrieved from PubMed and EMBASE (Figure 1).[5] After two sequential screens and a manual search, 64 clinical studies investigating clinical outcomes associated with PRBC storage duration were retained. Eight RCTs were removed, leaving 56 observational studies. Sixteen datasets were received from 14 investigators between January 2014 and January 2017. These studies covered 17,967 patients across burns, general surgery, ICU, oncology, acute medicine, trauma and cardiac surgery cohorts (Table 2). Overall, 77,962 units of PRBC were transfused across 16 studies with an average of 4.34 units transfused per patient. 8.1% of patients received more than ten units of PRBCs. The mean PRBC age transfused was less than ten days in 15.4% and at least 30 days in 18.9% of participants. The mean age of patients was 57.82, with 45.3% of patients being older than 65.

On paper level comparisons, included studies had similar recruitment dates, mean sample size (n=904.56 *versus* n=1003.53) and rates of positive mortality findings (36.36% *versus* 33.33%) compared to studies for which

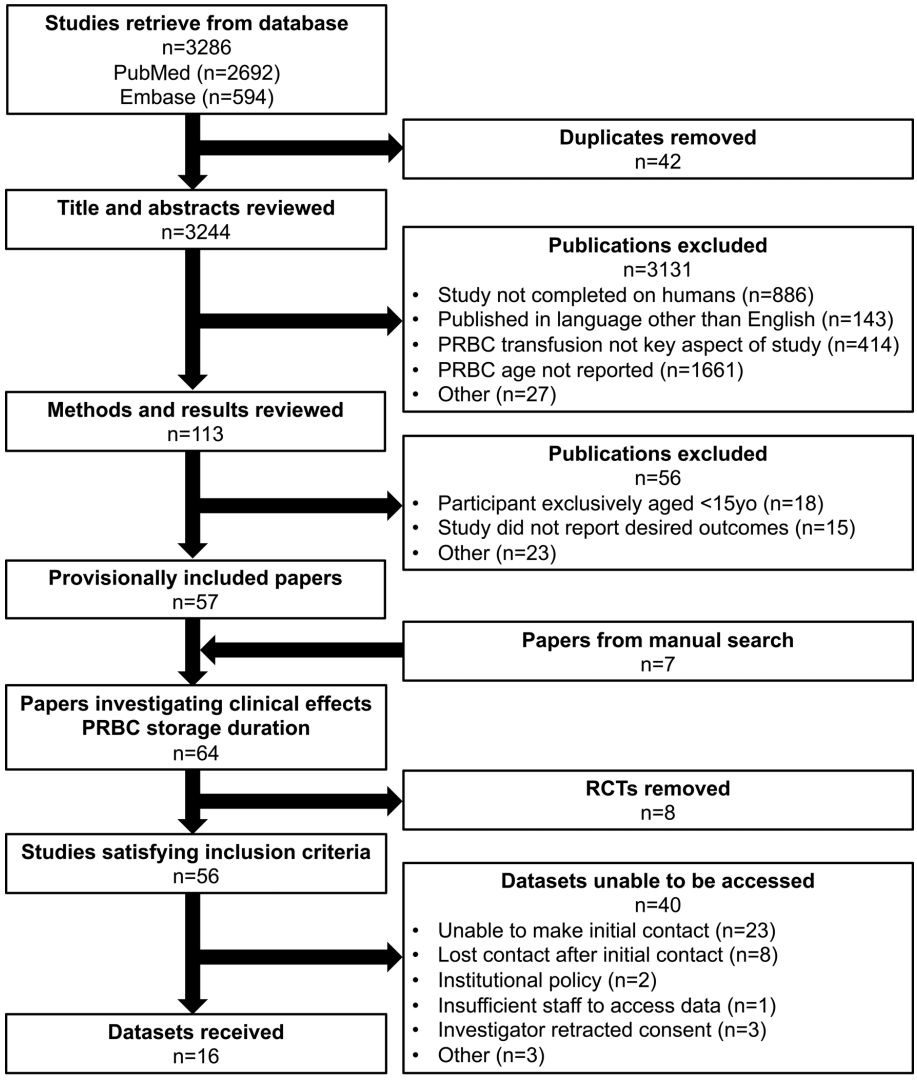

Figure 1. Outline of study selection. Fifty-six observational studies investigated the effects of PRBC storage duration on clinical outcomes, such as mortality, infection risk and hospital length of stay. Forty datasets were unavailable for various reasons: (1) no response from corresponding author after initial email, (2) lack of correspondence after initial contact, (3) institutional policy against data use by external investigators, (4) insufficient staff available to access data on site, (5) investigator retracted participation in study, and (6) other (e.g., data file corrupted). PRBC: packed red blood cells; yo: years old: RCT: randomized controlled trials.

datasets were unavailable (*Online Supplementary Table S1*). Received datasets included higher proportions of studies conducted in Europe (56.25% *versus* 25.00%) and ICU patients (31.25% *versus* 12.50%) compared to excluded studies. Unavailable datasets included higher proportions of studies conducted in the Americas (70.00% *versus* 25.00%) and trauma patients (32.50% *versus* 6.25%). Excluded studies also had increased rates of associations between PRBC age *versus* nosocomial infection (56.26% *versus* 12.50%) and HLOS (42.86% *versus* 25.00%).

There were significant variations in demographic, treatment and outcome variables released by investigators (*Online Supplementary Tables S2-S4*). Age (n=15), sex (n=14) and PRBC volume (n=16) transfused were the most consistently reported demographic variables across studies. Mortality (n=16), any infection (n=10) and HLOS (n=12) were the most commonly reported outcome variables.

Mean PRBC age >30 days associated with an increased risk of in-hospital mortality

Thirteen datasets contained the required covariate (age, sex, PRBC volume) and outcome data required for mortality analyses. These datasets covered 14,867 patients and 58,272 transfusions. There was no evidence of a significant association between mean PRBC age and post-transfusion in-hospital mortality (OR: 0.99, 95% CI: 0.98-1.00) (Figure 2). Similarly, there was no association between maximum transfused PRBC age and post-transfusion in-hospital mortality (OR: 1.00, 95% CI: 0.98-1.01, *Online Supplementary Figure S5*). There was substantial heterogeneity in both analyses with I^2 values of 63.6% and 59.0%, respectively. Sensitivity analyses demonstrated that effects estimates were similar across geographic location and patient subgroups (*Online Supplementary Figure S6*). Funnel plots for both analyses suggest that publication bias was not a significant concern. Time-lapse analyses demonstrated a trend of increasing OR in favor of fresh PRBCs from 1980-2011 (*Online Supplementary Figure S7*). Extremes analyses associated mean PRBC stored for at least 30 days with increased in-hospital mortality risk, as compared to mean PRBC stored for less than ten days (OR: 3.25, 95% CI: 1.27-8.29, Table 3). This association persisted in patients who received leukoreduced PRBCs (OR: 2.74, 95% CI: 1.39-5.36, Table 3).

No association between PRBC age and nosocomial infection

Eight datasets were incorporated in the nosocomial infection analyses covering 2716 patients. Mean PRBC age (OR: 0.99, 95% CI: 0.97-1.02, Figure 3) and maximum PRBC age (OR: 1.00, 95% CI: 0.96-1.04, *Online Supplementary Figure S8*) transfused were not correlated to nosocomial infection. There was moderate heterogeneity in both analyses with I^2 values of 42.5% and 28.6%, respectively. Stratifying analyses by geographical location and patient subgroup did not significantly alter effect estimates (*Online Supplementary Figure S9*). Funnel plots were not constructed as less than ten datasets were analyzed. Nosocomial infection ORs remained consistent across recruitment periods on time lapse analyses (*Online Supplementary Figure S10*). Extremes analyses did not iden-

Table 2. Demographic features of patients in aggregate dataset.

Demographic Characteristic	PRBC dataset (n=17,967)
Age (years; mean)*	57.82 ± 0.17
<40 years old (count, %)	3094 (17.2)
40-65 years old (count, %)	6425 (35.8)
>65 years old (count, %)	8146 (45.3)
Sex (male; count, %)	9865 (54.9)
Mean volume of PRBC transfused (units)*	4.34 ± 0.04
>10 units (count, %)	1462 (8.1)
Mean age of PRBC transfused (days)*	16.9 ± 0.12
<10 days (count, %)	2771 (15.4)
≥ 30 days (count, %)	3399 (18.9)
In-hospital mortality (count, %)	5010 (27.4)
Nosocomial infection rate (count, %)	675 (19.2)
Average HLOS	13.92 ± 0.15
<5 days (count, %)	3441 (18.8)
5-14 days (count, %)	7418 (40.5)
>20 days (count, %)	2790 (15.2)
Clinical setting	
Cardiac surgery (count, %)	4403 (24.0)
General surgery (count, %)	412 (2.2)
Acute medicine (count, %)	9967 (54.4)
Intensive care unit (count, %)	757 (4.1)
Orthopedic surgery (count, %)	871 (4.8)
Other (count, %)	1904 (10.4)
Geographic location	
The Americas (count, %)	1863 (12.5)
Europe (count, %)	7258 (48.8)
Middle East (count, %)	1695 (11.4)
ANZ (count, %)	4051 (27.3)

*All means presented ± standard error of the mean. ANZ: Australia and New Zealand; PRBC: packed red blood cells; HLOS; hospital length of stay.

Table 3. Odds ratios from extremes analysis for in-hospital mortality and nosocomial infection risk as a function of mean PRBC age. Patient age, PRBC volume and sex are entered as covariates in the logistic model.

Independent variable	Mortality		Mortality (LR)		Nosocomial infection	
	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
Mean PRBC age	3.25*	1.27-8.29	2.74*	1.39-5.37	1.57	0.39-6.27
Patient age	1.02*	1.01-1.03	1.03*	1.02-1.03	1.00	0.98-1.02
PRBC volume	1.03	0.98-1.07	1.02	0.97-1.07	0.97	0.86-1.08
Sex	1.14	0.90-1.45	1.16	0.92-1.47	1.99*	1.45-2.74

*=statistically significant (P<0.05). 95% CI: 95% confidence intervals; LR: leukoreduced patients only; PRBC: packed red blood cell.

tify any association between mean PRBC age and nosocomial infection risk (OR: 1.57, 95% CI: 0.39-6.27, Table 3).

No association between PRBC age and HLOS

Ten datasets were analyzed in the HLOS analyses covering 14,063 patients. The incidence rates were found to decrease by 3.1% and 0.4% for each additional day of mean PRBC age and maximum PRBC age, respectively. However, neither rate was statistically significant after controlling for age, sex and PRBC volume. There was significant heterogeneity in the mean PRBC age analyses with an I^2 value of 98.6%. Patient subgroup analyses identified ICU and other patients as major sources of heterogeneity compared to cardiac surgery patients (*Online Supplementary Figure S11)*. However, all patient subgroups groups generated similar effect estimates. The effect estimate for studies originating from the USA was significantly different to studies from Europe and other countries, however, there was only one study in the USA group (*Online Supplementary Figure S11*).Time-lapse analyses found that the incidence rate ratio remained stable over time (*Online Supplementary Figure S12*). On extremes analysis, the incidence rates for days in hospital increased by 8.3% in patients with a mean PRBC of at least 30 days compared to less than ten days. Similar to the general analyses, this rate was not statistically significant after adjusting for age, sex and PRBC volume transfused.

Discussion

The use of IPD enabled consistent treatment and outcome measures throughout analyses, leading to reduced variability and improved precision compared to paper level meta-analyses.[5] The association between stored PRBCs and mortality aligned with the results of observational, but not RCT paper level meta-analyses.[10,11,19] These RCT meta-analyses included small pilot trials and combined diverse populations. Furthermore, PRBCs also vary significantly within a blood bank due to donor, preparation and storage factors.[20] The increased variability and confounding factors may have obscured associations between PRBC age and mortality.[19] While large RCTs have not associated PRBC age with mortality, these RCTs did not test PRBCs at the end of shelf life.[7-9,21]

The extremes analyses addressed this issue by comparing patients transfused with a mean PRBC of at least 30 days to those transfused with a mean PRBC of less than ten days from observational studies. In this way, the pooled IPD analysis bridged the dissonance between clinical approaches (which tested stored PRBC aged 17 ± 13 days) and *in vitro* protocols (which sampled PRBCs over the course of shelf life).[22] The association between PRBC >30 days old and mortality differed from secondary analyses of the Informing Fresh *versus* Old Red Cell Management (INFORM) study.[23] In the secondary analyses, the use of maximum PRBC age to define stored PRBCs may have overestimated the aggregate PRBC age transfused.[24] Patients transfused with predominantly fresh PRBC units, but with the addition of one PRBC unit >35 days old could inflate the number of "survivors" in the stored PRBC group.

The finding that PRBC age was not associated with nosocomial infection corroborated the results from large RCTs.[7,21] However, the datasets available for IPD analysis had lower rates of associating PRBC age with nosocomial infections compared to unavailable datasets – potentially leading to an underestimation of the relationship between PRBC age and nosocomial infection risk.[19]

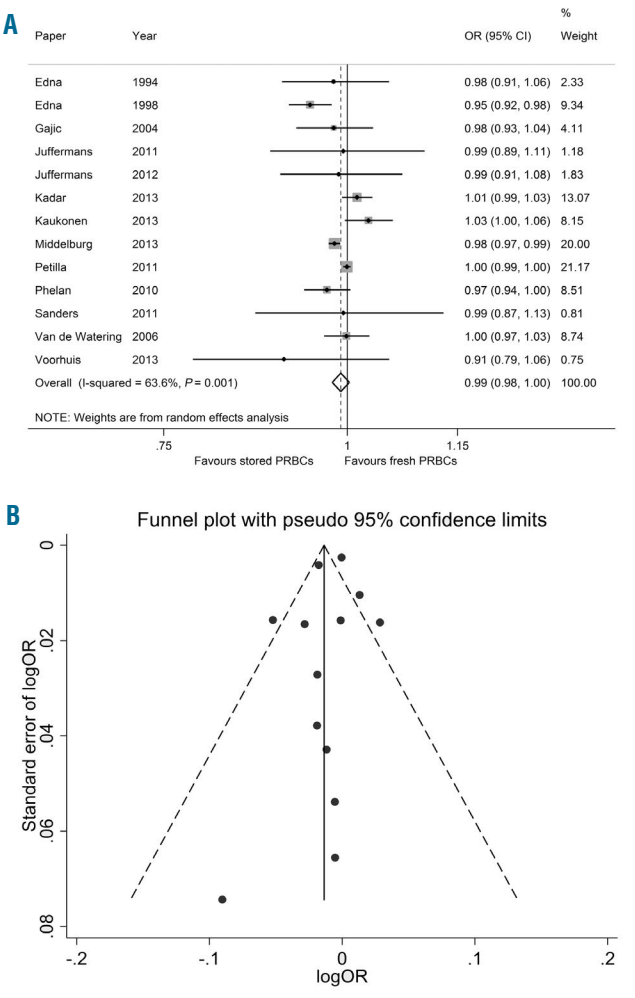

Figure 2. Forest plots and funnel plots for mortality analysis as a function of mean PRBC age. Mortality odds ratios were calculated for each study using logistic regression with mean PRBC age transfused as the independent variable. Age, sex and PRBC volume were covariates. (A) Odds ratios were then combined using random effects models. (B) Funnel plots were generated for each analyses to assess for publication bias. OR: odds ratio; CI: confidence interval.

There was no association between PRBC age and HLOS. This may have occurred due to substantial HLOS variability as it is a composite indicator of disease severity, treatment efficacy and safety, that is heavily modulated by social factors. This pooled patient analysis was the first assessment of HLOS across multiple clinical PRBC age studies. Published studies describing PRBC storage effects have reported HLOS as stratified count data,[25] median and interquartile range,[26-28] median and range,[29] mean and standard error,[30] and Pearson's correlation result[31] – making paper level meta-analyses difficult. The IPD analysis abrogated this issue by using patient level HLOS data, allowing one consistent measure across all studies.

The potential limitations of this study include long recruitment time, applicable to high-volume PRBC transfusion, potential confounding factors and selection bias. PRBC processing and transfusion guidelines may have

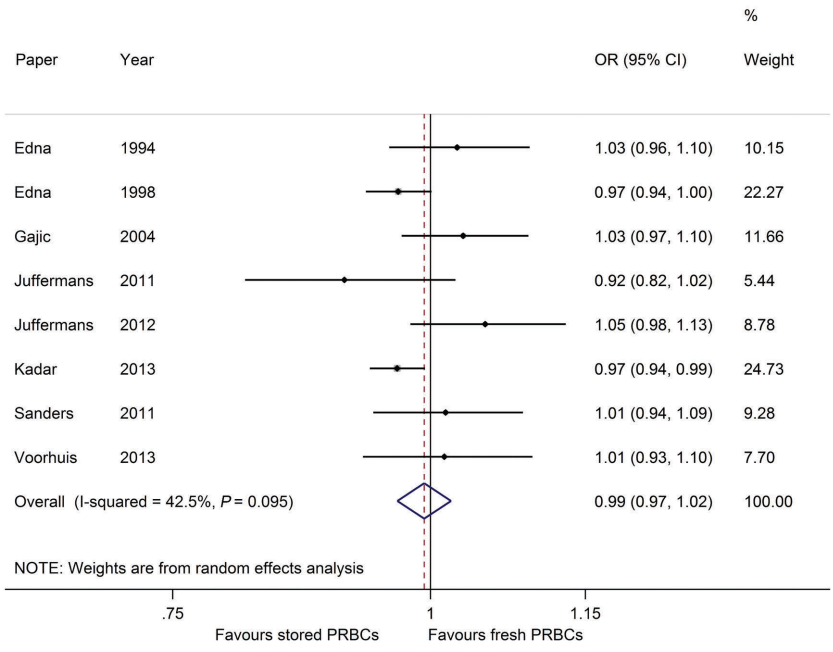

Figure 3. Forest plots for nosocomial infection analysis as a function of PRBC mean age. Nosocomial infection odds ratios were calculated for each study using logistic regression with mean PRBC age transfused as the independent variable. Age, sex and PRBC volume were entered into the model as covariates. Odds ratios were then combined using random effects models. OR: odds ratio; CI: confidence interval.

changed during the recruitment period (1980-2011), altering the effects of PRBC age on adverse events. Notably, the association between PRBC age and mortality persisted after leukoreduction – one of the major changes in PRBC processing over the past 20 years. The mean volume of PRBC transfused in this study was approximately four units, signifying that the results likely have limited applicability to massive transfusion protocols where more than ten units can be transfused in one incident.

This pooled patient data analysis may have been affected by confounding factors due to the use of observational studies, as patients were not randomly allocated to treatment groups. This pooled patient analysis incorporated three covariates – age, sex and PRBC volume, which have been demonstrated to be the most influential confounders over multiple studies.[32,33] The assessment of a limited subset of observational studies introduced the risk of selection bias. This effect was less likely to affect the observed association between PRBC age and mortality as it aligned with the results of existing meta-analyses.[11] Furthermore, included studies were similar to excluded studies in terms of study size, recruitment dates and rates of positive mortality findings.

Pooled IPD analysis found an association between PRBC >30 days and increased risks of in-hospital mortality. This result was significant as 24.5% of all O negative PRBC units are transfused after 35-42 days of storage in Australia.[34] These results align with the findings of *in vitro* studies and support the shortening of PRBC shelf life to 35 days, which has already occurred in some jurisdictions (The Netherlands, UK, Germany, China). Shortening PRBC shelf life should be approached with caution due to its implications for blood bank management, product wastage and PRBC access in remote locations. Ideally, these findings should be confirmed using extremes analyses of pooled patient data from recent large RCTs prior to implementing changes.

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